INTRODUCTION OF MICROBIOLOGY

Medical microbiology

- Role of micro – organisms in human health and diseases

1.Anton – von Leeuwenhoek - Animal cules

↓

Ground lenses

2.Edward Jenner - Small pox vaccine

3.Louis Pasteur

- Petrification of milk
- Germ
- Fermentation techniques
- Liquid culture to isolate organism

4.Joseph lister - Father of antiseptic surgery

5.Robert Koch

a. Used aniline dye for staining
b. Discovery of anthrax bacilli
c. Used solid culture to isolate organism
d. Koch postulates
e. Hanging drop for motility (used while trying to identify an organism)
f. Isolation of organism in true cultures

Exceptions of Koch's postulates

T. pallidum
M. leprae
These are animal model
But do not grow in- vitro

N. Gonorrhea - Does not have an animal model but can grow in vitro

6.Paul Ehrlich – father of chemotherapy

- a) Discovered acid fast nature of TB bacilli
- b) Arsenic compound for syphilis
- C) Side chain theory
- d) staining for cells
- e) interaction of toxin and antitoxin ↓ Ehrlich phenomenon

7. Alexander Fleming – penicillin
8. Christian gram – gram staining
9. Ronald ross – life cycle of mosquito
10. Ernst Ruska – Electron microscope
11. Barbara MC Clintock – transposons
12. Kary Mullis – PCR
13. Landsteiner – Blood grouping

MICROSCOPE

- Ernst Ruska – Electron microscope
- Bacteria are prokaryotes; hence nucleus is primitive i.e., No nuclear membrane. The respiration is through mesosomes
- Bacteria are measured in micrometers
- Fungi & protozoa – Eukaryotes is nucleus is well formed
- Viruses
  - Very small
  - Measured in nanometers
  - Visible in electron microscope
- Various microscope

Microscope

R	C	M
Good resolution → closely placed objects ↓ 2 separate images	Good contrast – staining	Good magnifications • Ocular lens • Objective lens Refractive index-RI Oil improves RI

Various microscope

1) Light filed microscope
   - Parts
     - Base
     - C- shaped Arm
     - Mechanical stage
   - Lenses
     - Ocular lens(10x)
     - Objective lens
     - Movable nosepiece 4x,10x,40x,100x
   - Illumination
     - Source of light – bulb
     - Condenser – cone of light
     - Iris diaphragm – controls the light enters the condenser
     - Coarse & fine adjustment

2) Dark filed microscope
   - Dark filed microscope

     ↓

     Light does not enter the objective directly but enters peripherally

     ↓

     Light that is reflected by the specimen enters the objective lens
   - Uses
     - Used for unstained living cell
     - Used for thin coiled organism e.g., spirochetes

3) Phase contrast microscope

- Contrast is achieved by

  ↓

  Different in the cells & water

- Uses

  - Used for unstained living cells
  - Motility of organisms (to study)
  - To study the endospores & inclusion bodies

4) Fluorescent microscope

- Here the fluorescent dyes get exited in the UV light

  ↓

  Fluorescence

- Illumination

  ↓

  Excitation filter

  (Only allows short wavelength of UV light to pass through)

  ↓

  Dichromatic mirror

  (This UV light is projected on the specimen coated with fluorescent dye)

  ↓

  Specimen

  ↓

  UV of short wavelength to make a visible longer wavelength ray

- Uses
  - Autofluorescence
  - Organisms where there are autofluorescence's it is used to identify the presence of Cyclospora
  - Dyes are used to coat micro-organisms
  - Acridine dye- malarial parasite
  - Auramine dye – mycobacterium TB
- Autoimmunity

  IFA – used to detect antigens or antibodies bound to antigens on a cell
  - ANA – SLE
  - C- ANCA
  - P- ANCA

5) Electron microscopes

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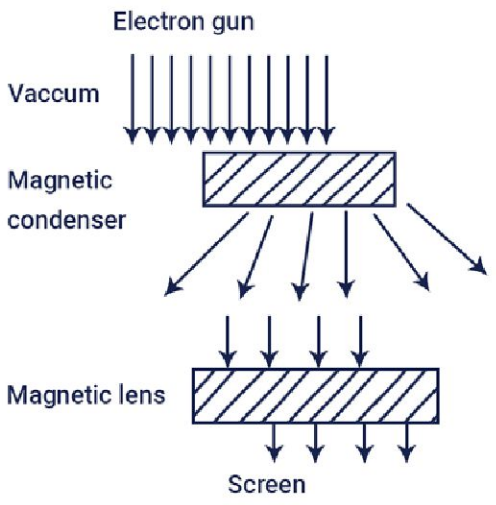
Transmission microscope	**Scanning microscope**
• Preparation – Fixation – Glutaraldehyde – Dehydration- Ethanol – Embedding – plastic polymer – Slicing – Microtome Knife is used for slicing – Mounting – copper → slide	• Used to study surface of micro-organisms


Electron gun
Vaccum
Magnetic condenser
Magnetic lens
Screen

- Electron → used to study structure of viruses
- Microscope → used to study cell organelles
- Staining – Heavy metals- lead citrate
- Negative staining – Phosphotungstenic acid

↓

Dark background

Not absorbed by the organisms

- Freeze – etching technique

  - Here the specimen is cobbled, which is exposed to liquid nitrogen

↓

Sublimation

↓

Shadowing with platinum & other heavy metals

↓

Transmission electron microscope

CLASSIFICATION OF MICROBES

Culture media

1) H_2O_2
   - Decrease mineral content
   - Produce hydrogen & oxygen ions
2) Nacl
3) Peptone – partially digested protein
4) Agar Agar – solidifying agent

   ↓
   - Biologically inert
   - It solidifies at 42°C & melts at 98°C
   - Obtained from sea weeds - Gelidium, Gracilaria
   - Long chain polysaccharides & some salts like Ca^{2+} & Mg^{2+}

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5) Basal media – used for non-fastidious organisms
   - It is a base for other media
   - Used to study bacterial growth curve
   - Used to study colony morphology
   - To identify pigment production

   Example: - Peptone water – peptone + Nacl + H_2O_2
   - Nutrient broth – peptone + meat extract
   - Nutrient Agar – Nutrient broth + Agar (1-2%)
   - 1-2% - solid Agar
   - 0-5%- semi solid Agar (used to study motility of organisms)

- 6% – used to study proteus
- Proteus produce a phenomenon known as swarming which is inhibited if the % of Agar is increased

6) Enriched media

- Basal media + Enrichment (Blood, serum, egg)
- Blood Agar – add blood (Sheep, Horse, Human)

↓

Added at 42°C

↓

Blood Agar (basal + Blood)

- Chocolate Agar – blood added at 70 – 80°C

↓

RBC is lysis happens

↓

Releases factors

H. Influenzae grows in chocolate Agar

- Loeffler's serum slope

7) Enrichment media – liquid media

- Allows certain organisms to grow
- Inhibit certain organism

- Selenite F both – Salmonella
- Gram negative broth – Shigella
- APW – V. Cholerae

8) Selective media – solid media inhibits growth of commensals

- XLD
- TCBS
- PTA – C. diphtheria

- LJ medium – M. Tuberculosis
  ↓
  Malachite green → Inhibits the growth of commensal flora

9) Transport media – to maintain the viability of the organism
  - Autoclaved salt water
  - Venkatramana – Ramakrishnan medium

- Bacteria – able to isolate in artificial culture media
- Virus-unable to isolate artificial culture media as they do not grow in them
- Fungi – can be isolated in
  - SDA
  - Bird seed Agar
  - Corn seed Agar
- Some fungi are non-culturable
- Fungi has – Chitin] Differentiates from other organisms
  – Ergosterol

Bacteria

a)Cocci

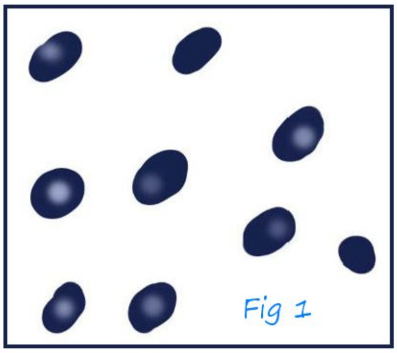
Fig 1

b) Bacilli

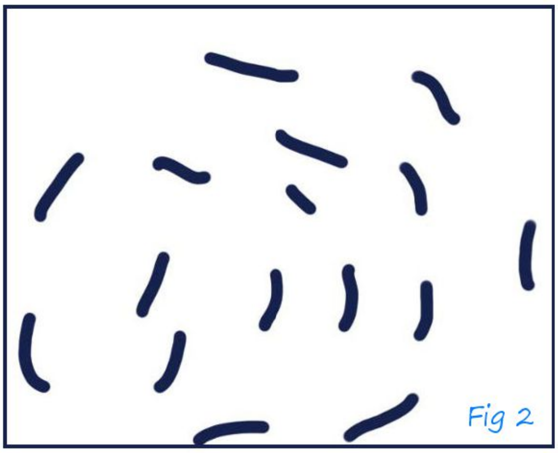

Fig 2

c) Spirochaetes

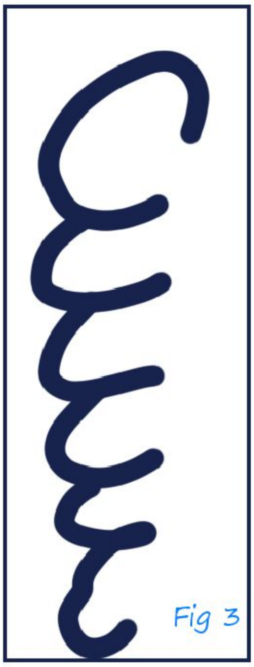

Fig 3

GENERAL MICROBIOLOGY

Bacterial growth curve

i) Generation time: It is the time taken by the bacteria to produce 2 daughter cells in optimum condition

ii) Total count: contains both live + dead cell in specimen

iii) Viable count: live or viable cell in specimens

Growth curve –

- Multiplication of bacterial cell plotted against time
- 4 phases
  - a) Lag phase
  - b) Log phase
  - c) Stationary phase
  - d) Phase of decline

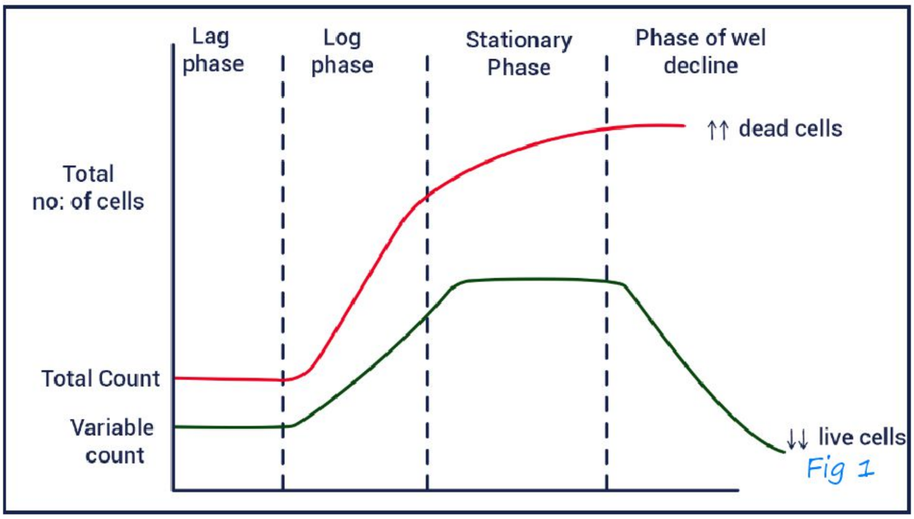

Fig 1

a)Lag phase – Time from inoculation

↓ Lag phase

Bacterial multiplication

- Bacteria is at its largest size
- Metabolites & enzymes acquits

b)In log phase

- Graph
  - straight line
  - Exponential growth
- Bacteria – small in size
- Best time to test for biochemical reaction
- Gram staining – uniformly stained

c)In stationary phase

- Bacterial growth starts to decrease because of
  a. Decrease in nutrients
  b. Increase in accumulation of end products
- Number of progenies = Number of cells dying
- Changes in bacteria
  - Sporulation occurs
  - Storage granules are seen
  - Bacteria produces exotoxins

d) In phase of decline

- Bacterial growth stops
- Viable count decreases
- Total count increases
- Changes in bacteria
  - Involution forms

BACTERIAL SPORES

Bacterial spores

- In dormant stage when the nutrient decreases spores consist of an innermost core (DNA), cortex, coat & exosporium (outermost)
- Sporulation – spore is formed in a vegetative cell
- Germination – vegetative cell from a spore
- Endoscope – spore is formed inside mother cell

Spore formation

- In a vegetative cell, an endoscopic is formed whenever there is a decrease in exogeneous nutrients
- Bacillus & clostridium – important

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Nutrient derivatization

- When the genetic DNA of bacteria, when it replicates, it forms an axial filament
- This is followed by the formation of a septum from outside to inside responsible for the formation of forespore
- Inner membrane engulfs the forespore & forms a cortex & coat around it
- The dipicolinic acid helps to stabilize the proteins and DNA in the endoscope
- The cortex is a thick peptidoglycan layer.

- Now, the vegetative bacteria tend to produce lytic enzymes and because of these, it ruptures, matures and ruptures from the mother cell
- This is how the spores are being released to the environment
- Difference between bacterial and fungal spore

Bacterial spore	**Fungal spore**
• Does not involve replication	• It involves replication

Demonstration of spores

- Gram staining – seen as refractive body
- Modified AF staining – 0.25% H_2SO_4→ it decolorizes
- Staining
  - Schnaffer – Fulton stain
  - Moeller stain

Sporicidal agents

1) E- Ethylene oxide
F – Formaldehyde
G – Glutaraldehyde
H – Hydrogen peroxide
O – Ophthalmic acid
P – Per acetic acid
A – Autoclave
H – Hot air oven
P – Plasma sterilization

Application

- These are used as biological indicator

• Geobacillus	• Non – toxigenic strains of clostridium
• Autoclave	• Hot air oven

- **Bioterrorism** – B. Anthracis

SIMPLE STAINING

- Staining provides contrast against
- Stain – it is an organic compound
- Benzene + chromophore + auxochrome compound

↓

To bind to tissues or fibers

- Basic stains – cytoplasm of bacterial cells. Takes in +ions
- Types of staining
  - Simple staining – (1 type of stain)
  - Different staining – (2 types of stain)

Staining

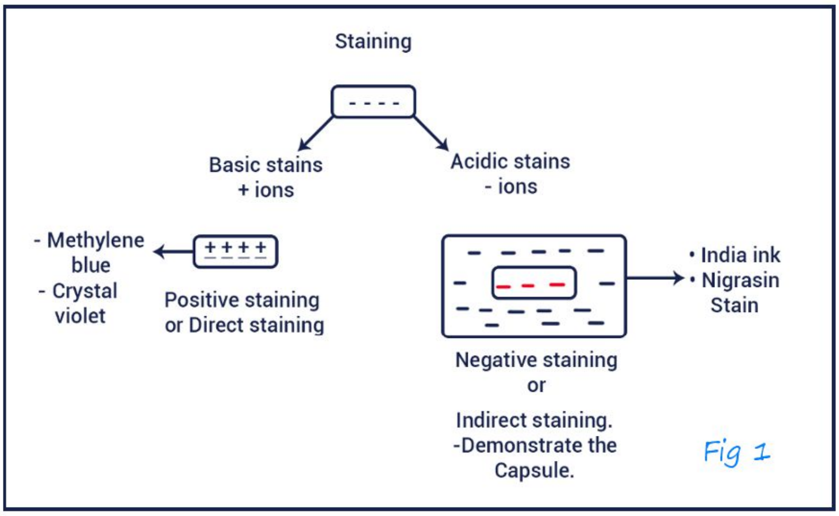

Staining
- - - -
Basic stains
+ ions
Acidic stains
- ions
- Methylene blue
- Crystal violet
+ + + +
Positive staining or Direct staining
Negative staining or Indirect staining. -Demonstrate the Capsule.
• India ink
• Nigrasin Stain

Fig 1

Fixation

- Heat fixation – can see morphology
- Chemical fixation – can see morphology

+

Internal structures

Others

- It kills the bacteria
- Smear gets adherent to the slide
- It makes it receptive to staining

Simple staining

- Methylene blue
- Crystal violet
- Gentian violet

- All bacteria take up the same colour
  Urethral swab – gonococcus
  (It is simple stained)

NEGATIVE STAIN

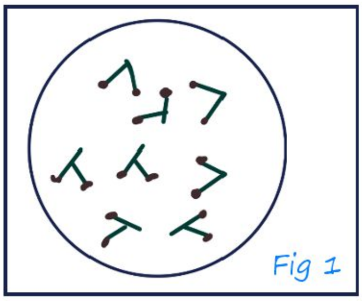
- Albert staining – different staining
  - C. diphtheria
  - Chinese letter pattern arrangement → can see green

Bacilli which have metachromatic granules

↓

Greenish black colour

C. Xerosis
Gardenella Vaginalis
– Metachromatic granules



Vital staining

↓

Differentiate living from dead cells

Living cells
↓
Exclude the stain

Dead cells
↓
Do not exclude stain

- Supravital staining
  - Cells are taken out of organism and dye is introduced
- Intravital staining
  - Dye is introduced into the body

GRAM STAINING

- Discovered by Christian gram
- Steps of gram staining: fixation of smear

Prime **M**inister **D**iscusses About **C**hina	Primary staining ↓ Mordanting ↓ Decolorization ↓ Counter staining	Gentian violet Crystal violet ↓ Mordanting (Gram iodine lugols iodine) ↓ De-ethanol acetone ↓ Saffrinine

Gram staining

PH theory

Gram positive organisms are acidic in nature

↓

Take up the basic dye

↓

Bacterial cytoplasm is acidic (It is easier for basic dye to bind to it)

Peptidoglycan

GPC

↓

Peptidoglycan layer - which is thick

↓

1° – crystal violet

↓

Mordant (dye - iodine complex)

↓

Decolorization (Reduces size pores of peptidoglycan

↓

Alcohol

↓

Counter stain (C-S) (It is not taken up)

GNB

↓

Peptidoglycan layer

- Thin
- Lipopolysaccharide

↓

Lipids

↓

crystal violet

↓

Mordant

Dye – iodine complex

↓

Decolorisation

↓

Lipopolysaccharide

↓

o o o o→ size of the pore increases

↓

Primary stain goes away & counter stain is taken up

Gram staining importance

- Morphology
- Emperical thereapy
- Fastidious organism – H influenzae
- Yeast- candida
- Ancerobic organisms – clostrodium

Modifications

- Kopeloff beerman's
- WEIGERT'S
- Jensen's
  - C. S – Neutral red
  - Gonococcus, Meningococcus
- Preston & morrels

Acid Fast Staining

- Introduced by Paul Ehrich
- Modified by both – Ziehl – Neelsen
- Acid fastness – attributed to the presence of mycolic acid in cell wall

Mycolic acid

↓

Mycobacteria → Resists decolorization by acid alcohol

Steps of staining Fixation

↓

Primary stain used is strong carbol fuchsin

↓

Decolorization – H_2SO_4 is used

↓

Counter by methylene blue

Microscopic picture

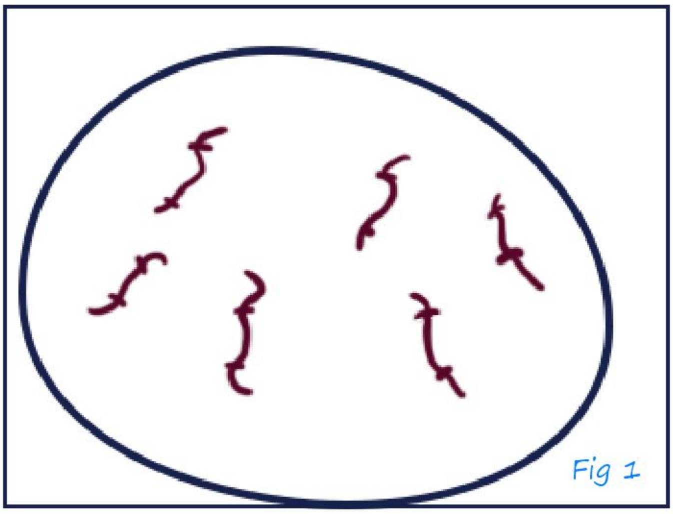


Fig 1

H_2SO_4 % Varies

- M. TB – 25%
- M. Leprae – 5%
- Nocardia
- Cryptosporidium – 1%
- Isospora
- Bacterial spore – 0.25% - 0.5%

AFB staining – NTEP

1.Inexpensive
2.Easily available – Advantages
3.Less expertise needed
4.To understand the infectivity of the patient
5.Limitation

- Cannot assess the response to treatment based on staining

↓

Treatment – culture – Gold standard

Cold staining– kinayn's method

↓

Acid alcohol – decolourised
Malachite green – counter staining

SILVER IMPREGNATION STAIN

- Spirochaetes
  - Borelia
  - Leptospira
  - Treponema

  ] Cannot be visualized under microscope
- Bacterial flagella
  - Responsible for locomotion

Hence, silver salt is used to impregnate, which increases the thickness of the bacteria, so that it can be visualised under microscope.

- Spirochetes
  - Levaditi method
  - Fontana method
- Flagella
  - Ryu's staining
  - Leifson's staining

PHYSICAL METHODS OF STERILIZATION AND DISINFECTION

Sterilization: 00:00:30

A. Sterilization - Devoid of organism and spores

it ↓ 10^6 CFU

(Colony forming units)

- Physical
- Chemical

B.Disinfection

- It ↓ses to, 10^3 CFU
- Removes spores
- Refers to
  - Inanimate objects
  - Chemical methods

C.Asepsis

- Refers to chemical sterilant and direct application

D. Decontamination

- Bacteria forming colony is reduced to 1 log of CFU

Factors affecting the efficacy of disinfection: 00:03:48

1.Organism

- Load of the organism

2.Nature of organism

- Highly resistant – Prions > cryptosporidium oocyst > bacterial spore > mycobacterium > parasitic cyst > non –

enveloped viruses >trophozoites > GNB > fungi > non – enveloped viruses > $\boxed{\text{enveloped viruses}}$

↓

Most susceptible

3.Nature of chemical disinfect

4.Concentration of chemical disinfect

5.Minor factors

- Duration
- Temperature
- Local environment
  - PH
  - Organic matter
  - Biofilms

Various methods of sterilization: 00:09:40

1.Physical method

a. Drying

b. Heating

c. Filetration

d. Radiation

e. Ultrasonic vibration

Drying: 00:12:10

- All bacteria have 70-80% of water
- Removing water causes delirious effect on bacteria
- Not a consistent method
- May not have great significance in spore eradication

Heating: 00:13:00

- Heating
  - Dry heat
  - Moist heat
- Dry heat
  - Proteins get degraded
  - Oxidative damage
  - Increase / elevated electrolyte
- Moist heat
  - Degradation as well as coagulation of proteins
- Various methods of dry heat

  1.Flaming
    - Loops – red hot

  2.Incineration
    - Temperature - 870-1200°C
    - Biochemical waste – yellow bin
    - Advantage - Reduces the amount of waste – ash

3.Hot air oven
  - It is an equipment which is electrically heated, consists of fan and thermostat
  - Temperatures (Holding temperature) – 160°C for 2 hours
  - Things that can be sterilized

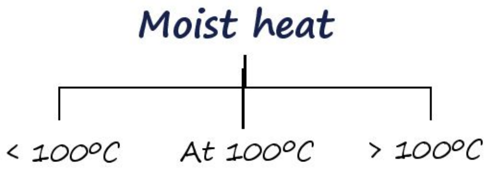
    a. Glassware – flask, petridishes

    b. Glove powders, glycerol

    c. Surgical instruments – scalpels

- Plastics should not be sterilized by hot air oven
- Precaution
  - All the equipment's are cotton plugged
  - Should be dry
  - Placed in a non-crowding way
  - Let it cool for 2 hours → Immediately opened glass ware might crack due to sudden change temperature
- Hot air oven controls
  - Biological indicator
    - B. Subtilis niges
    - Thermocouples
    - Brown tubes

Moist heat: 00:24:50



A. Below 100°C

Pasteurization

Holder's method

- 63° for 30 min
- Coxiella burnetti is an exception

Flash method

- 72° C for 2 min
  - Rapidly cool it less than – 13°C

B.At 100°C

a. Boiling
  - Spores not killed

b. Tyndalisation

c. Steaming

C.Above 100°C

- Autoclave

Inspissation (fractional sterilization)

- Sterilize egg and serum media
- Temperature – 80°C -85°C for about 30 min
- LJ medium and Loeffler's serum slope

Tyndallisation

- It happens at 100°C for 20 min
- Same as inspissation

- The process in both inspissation and tyndalisation sterilized for 3 successive days
  - 1st day – vegetive form (VF)
  - 2nd day – spores germinate to VF – killed
  - 3rd day – Remnant spores to VF – killed

Steaming: 00:32:35

- DONE IN Koch's steam sterilizer or Arnold's sterilizer
  - Temperature – 100°C for 90 min

Autoclave: 00:34:16

- Principle
  - Water boils when vapor pressure = Atmospheric pressure
  - ↑ atm pressure → ↑ Boling point
- Pressure in a closed vessel is increased

↓

Temperature at which water boils also increases

Sterilization

a. Surgical instrument
b. Culture media
c. Plastics
d. Glass ware
e. Biomedical waste

- Things that cannot be sterilized

  a. Water labile - Glove powder, glycerol
- Autoclave: Holding temperature 121°C for 15 min and 15 PSI
  - At 133°C for 3 min and 30 PSI

Control

- Biological indicator - Geobacillus stearothermophilus
- Chemical indicator - Thermocouples

Water bath: 00:40:00

- Usually done for serum and vaccines sterilization

Summary of moist heat: 00:40:45

Moist heat

Below 100°C	At 100°C	Above 100°C
• Water bath	B- Boiling	Autoclave
• Inspissation	T- Tyndalisation	
• Pasteurization	S- Steaming	

Filteration: 00:41:55

- Done for heat labile substances

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A. Depth filters
  - Based on thickness and density of filters
  - Ex: Candle filter, asbestos filter, sintered glass filters

B. Membranous filters
  - Depends on pore stage
  - 0.22 nm
  - Anything above the pore size remains on the membranes of the filter
  - Controls

    a. Brevundimonas

    b. Serratia

Filteration of air: 00:44:38

A. HEPA filters

- Fitted in biosafety cabinets
- Remove 99.9% particles of size 0.3 nm

B.ULPA filters

- 0.12micro meter are removed
- Efficacy – 99.99%

Ionization radiation: 00:46:25

Ionizing radiation	Non-ionizing radiation
• Gamma rays • Cosmic rays • Mechanism: damages DNA ↓ Without increasing temperature (Cold sterilization)	• UV rays ↓ 250-300nm wave length • It can damage eyes and skin • Done for sterilization of OT • Used in laminar air flow hood

- Materials sterilized
  - a. Plastics
    - Catheter
    - IV sets
    - Syringes
  - b. Catgut
  - c. Implants
  - d. Grafts
  - e. Adhesive dressings
- Advantages
  - Highly penetrative
  - Does not ↑se temperature
  - Acts very rapidly
- Biological indicator
  - Bacillus pumilis

Ultrasonic vibration: 00:51:00

- High frequency vibrations affecting bacterial cells
- Not reliable
- May not work on spores

CHEMICAL METHODS OF STERILIZATION AND DISINFECTION

Chemical disinfection 00:00:26

1. Alcohol – MOA – denaturation of protein
   - They are bactericidal, fungicidal, can be effective against enveloped viruses
   - It is not sporicidal
   - Commonly used
     - Ethyl alcohol
     - Isopropyl alcohol – 70% - 80% - used for sterilization of thermometer
2. Aldehydes
   - It is sporicidal
   - MOA – binding to nucleic acid and cell proteins
   - Commonly used
     - Glutaraldehyde
     - Formaldehyde
     - Ortho-phthaldehyde (OPH)

a. Formaldehyde

- Formalin dissolved in water and alcohol
- Use

i. Preservation of specimen

ii. Fumigation

- Disadvantages

i. It is an irritant

ii. It is carcinogenic

b. Glutaraldehyde

- 2% cidex
- Less irritant and odor is better
- This is more commercially viable
- Activation is by alkalization
- Active only for 14 days
- Some activity is seen against mycobacteria

c. OPH

- It does not require activation
- It is not corrosive
- Less irritant
- Can have activity over mycobacteria
- It is more stable
- Uses –Sterilization of bronchoscopes and endoscopes

Phenols 00:09:41

- (Carbolic acid) ← Joseph lister
- MOA – denaturation of proteins
- Advantages

i. ↑↑ mycobacterial activity

ii. The efficacy is unaffected by organic matter

iii. Phenol – 2% cresol – used to disinfect sputum sample before discarding

- 2 categories

A. Disinfectants

  - Lysol
  - Cresol
  - xylenoc

B.Antiseptis

- Savlon – Chlorhexidine

  +

  cetrimide
- Dettol – Chlorxylenol

  ↓

  Active agent

Halogens 00:14:25

Halogen

Iodine

Chlorine

Iodine

Tincture iodine

↓

- Alcohol or water based of KI
- Disadvantage

a. Staining of skin

Iodophore

↓

Iodine with a carrier

↓

"Povidone"

- Advantage
  a. Water soluble
  b. Does not stain skin

- Chlorine
- Uses

i. Disinfection of swimming pools
ii. Food industry
iii. Disinfection of lab
iv. Bleaching agent to remove stains

- Commonly used
    - Chlorine gas
    - Sodium hypochlorite
    - Calcium hypochlorite
- MOA – It forms hypochlorous acid
- Disadvantages

i. It is corrosive

ii. It is carcinogenic

iii. It is not stable

↓

Prepared everyday

Heavy metals 00:09:55

Mercury	Copper	Silver
↓ Thiomersal ↓ Vaccines	↓ $CuSO_4$ ↓ • Fungicidal • Prevents over growth of algae	↓ • Silver sulfadiazine ↓ Burns • Silver nitrate – Used in 1% concentration – Used in ophthalmia neonatorum

- MOA – This bind to sulphydryl groups and inactivates them

Surface acting agents (Surfactants) 00:23:25

a) Cationic surfactant

- Eg: Quaternary ammonium compounds
- MOA → It disrupts the cell membranes and denaturation of proteins
- Advantages → Effective against GPC and GNB
- Not mycobacteriocidal, sporicidal
- Disadvantage – Inactivated in acidic p^H

b) Anionic surfactant

- Eg: Common soaps
- It is most effective in acidic p^H

c) Amphoteric surfactant

- Eg: Tego compounds
- It is effective at variable p^H

Dyes 00:28:40

Aniline dyes	Acridine dyes
a. Gentian violet	a. Euflavine
b. Crystal violet	b. Proflavine
c. Malachite green	c. Acriflavine
– selective agent in LJ medium	

- Advantages – has effect on GPC, GNB
- No effect on mycobacteria

Oxidising agents 00:31:34

Hydrogen peroxide (H_2O_2)	**Peracetic acid**
• MOA _ Breaks off to release hydroxyl free radicals ↓ Affects the DNA and lipids of organism • Conc – 3 – 6% and 10% in spores • Uses - T - Tonometer biprisms - V – Ventilators - S – Soft lenses • Advantages - Not carcinogenic - Not irritant - Environment friendly - Does not adhere on tissues	• More potent than H_2O_2 • Uses i. Plasma sterilization ii. Sterilize hemodialyzers

Plasma sterilisation 00:37:33

- Plasma is considered as 4th state of matter
- Take enclosed chamber → Create vaccum with radiofrequency or microwave

↓

Excites gas molecules

↓

Forms free radicals (Unpaired electrons and are highly reactive)

↓

They interact with cell components – nucleic acid and enzymes

↓

Metabolism of the organism is disrupted

- This takes place at normal temperature
- Plasma sterilization

Evacuation of the chamber

↓

H_2O_2 is introduced and is vapourised

↓

H_2O_2 is passed over all the equipment

↓

Radiofrequency – Gas plasma

Or

Microwaves

↓

Free radicals (unpaired electrons)

↓

Nucleic acid, enzymes

↓

Disrupts the mechanism

Gaseous sterilisation 00:45:16

A. Ethylene oxide

- Advantage
  1. i. High penetrating power
  2. ii. Passes through the packaging material
- Disadvantage
  - i. Highly inflammable (inert gas)
  - ii. Carcinogenic
  - iii. Environmentally friendly
  - iv. Aeration
- Sterilization control – Bacillus Globigi
- Factors that affect this method
  - a) Conc. of ETO – 700 mg/L
  - b) Humidity – 50-60%
  - c) Temp – 38°C
- Heart lung machine, catheter, suture, petridishes are sterlised by this method

B. Low temperature steam formaldehyde

- Fumigation of OT lab's
- KMO_4 with formalin
- Disadvantages
  - Irritant
  - Sealing of room for 48 hours

C. BPL (0.2%)

- Disadvantages
  - Low penetrating power
  - Carcinogenic
- Uses
  - Confined to vaccine

Sporicidal agents 00:52:22

- E – Ethylene oxide
- F – Formaldehyde
- G – Glutaraldehyde
- H – Hydrogen peroxide
- O – Ophthalic acid
- P – Peracidic acid

- Various methods by which spores can be destroyed
  - A – Autoclave
  - H – Hot air oven
  - P – Plasma sterilization

Biological indicators 00:54:06

- Humans – Hot air oven → Cl. Tetani and B. Subtilis niger
- Practice – Plasma sterilization → B. Stearothermophilus
- Artificial – Autoclave → B. Stearothermophilus
- Intelligence – Ionising radiation → B. pumilus
- For – Filtration →Brevindimonas and serratia
- Everything – Ethylene oxide → B. Globigi

Testing of disinfectant 00:58:05

1.Rideal walker test

- Disinfectant ability to kill S. Typhi in given time
  Phenolic compound in the same time
- >1 – disinfectant is more effective than phenolic count
- Disadvantahes:
  - Tests only against phenol compounds
  - Does not test efficacy in the presence of organic matter

2.Chick martin test

- In the presence of organic matter

3.Capacity test

- Capacity of the disinfectant to work in increasing microbiological load

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4.In – use test

- Ability of the disinfectant to destroy or kill a specific CFU of staphylococcus

- **Spaulding's classification**

level	Disease risk	Process	Materials
Critical	High	Sterilization: Autoclave, chemiclave. dry heat or immersion in full strength glutaraldehyde (8 hours for sterilization and 30 minutes for high-level disinfection	Items used in surgery that pierce soft and hard tissue, such as scalpel blades, burs, forceps, elevators, needles, files, bone rongeurs, periodontal instruments, surgical drains and any other instrument used in surgical sites, dental explorers and probes, biopsy punch
Semicritical	High	Sterilization: Autoclave, chemiclave, dry heat, immersion in full strength glutaraldehyde (8 hours for sterilization and 30 minutes for high-level disinfection	Items that do not necessarily penetrate soft and hard tissues but which cross the vermilion border into the oral cavity, such as mouth mirrors, handpiece, anesthetic syringes, chip syringes. impression trays and spatulas
Noncritical	Moderate	Surface disinfection: Phenolics, iodophors, quatemary ammonia compounds Sanitization: Scrub wash with soap and water Barriers: Impervious barriers	Items used in dentistry which do not cross the vermilion border or penetrate the soft tissues, such as chair light handles, instrument trays, high touch work surfaces, bracket tables, chair controls, air/ water syringes, hoses and dental chairs
Environmental	Mode	Disinfection: Intermediate to low level	Table and counter surface, floor, door handles

INTRODUCTION TO MEDIAL MYCOLOGY

- Medical mycology – causes mycoses
- Fungi
  - Eukaryotic
  - Cell wall – Chitin, β D glucan
  - Cell membrane – Ergosterol
- Antifungals
  - Mode of action

β Glucan	**Ergosterol**	**DNA synthesis**
↓ Echinocandins (They inhibit β glucan synthesis)	↓ A- Alylamines A – P – Polygenes (A, P – inhibit ergosterol)	↓ Consists of antimetabolites ↓ Flucytosine (inhibits DNA synthesis)

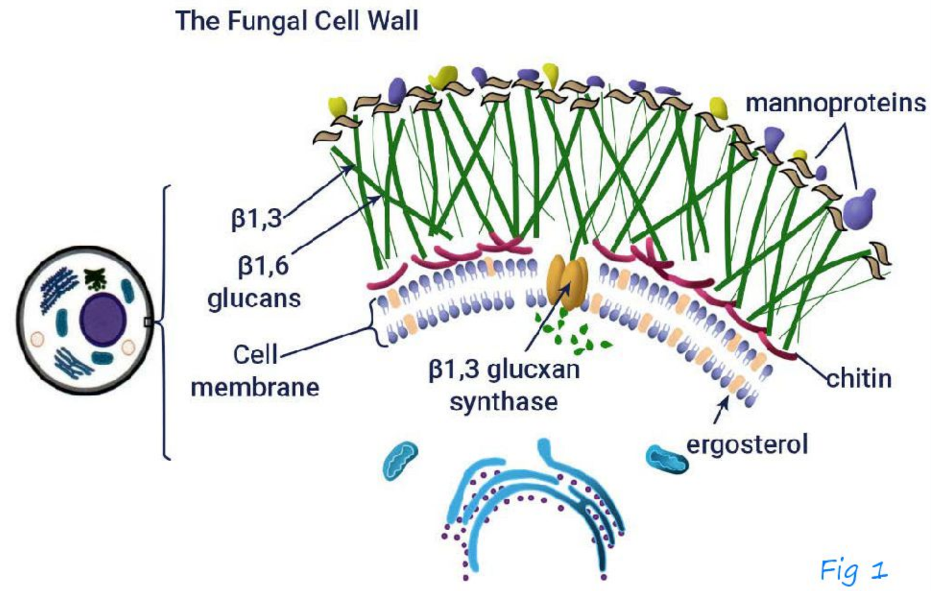

The Fungal Cell Wall
mannoproteins
β1,3
β1,6
glucans
Cell membrane
β1,3 glucxan synthase
chitin
ergosterol

Fig 1

- β- Glucan test is non- invasive test that is developed for invasive conditions like invasive like invasive aspergillosis
- Cryptococcus & Zygomycetes → they do not produce β Glucan

Classification

I) Morphological

II)Taxonomical

III) Clinical condition

I) Morphological classification

- Yeast (True yeast)

  e.g., Cryptococcus, Sacromycetes
- Yeast like

  Constriction is a characteristic feature
- Molds – branching filament are seen

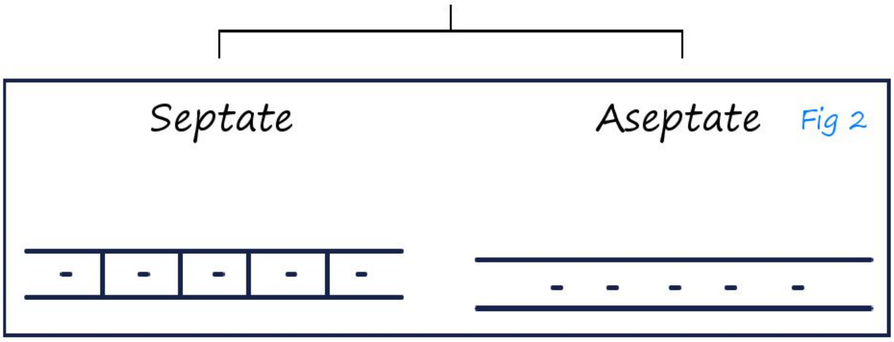

Septate
Aseptate

Fig 2

- Mycelium – mass of filaments

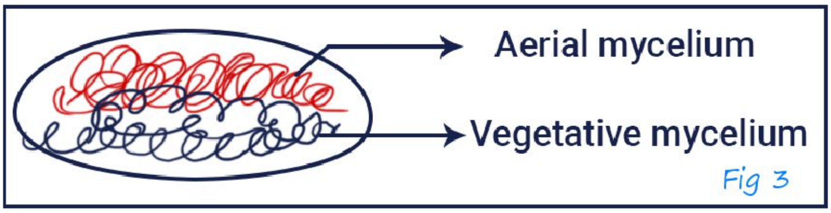

Aerial mycelium
Vegetative mycelium

Fig 3

- Dimorphic fungi
  - At 26°C hyphal forms
  - At 37°C yeast forms

II)Taxonomical classification: sexual spores

Zygomycota	Ascomycota	Basidiomycota	Deuteromycota
↓ Zygospores ↓ Aseptate hyphae E.g., mucor Rhizopus	↓ Ascospores ↓ septate hyphae Aspergillus	↓ Eg: Cryptococcus	↓ Fungi imperfecti • medically important fungi • sexual state is not defined

III) Clinical classification

Superficial mycosis	**Subcutaneous mycosis**	**Systemic mycosis**	**Opportunistic mycosis**
It involves • Skin • Nail • Hair • Mucosa	• It involves skin with subcutaneous tissue • Direct inoculation	Multiple organ involvement	Commences causes infections

Lab diagnosis

Routine microbiology	Histopathology	
• KOH mount – Skin – 10% – Nail, – Tissue – 20-40% • India INK preparation, Nigrosin – Negative staining (here, the background is stained) • Calcofluor white	PAS stain ↓ Only stain live fungi	GMS stain ↓ Stains both live & dead fungi

Culture

- Grown on SDA (P^H on SDA – 5.6%)
- (P^H can vary to 7.2% – Emmon's modification)

Others

- BHI Agar
- Corn meal Agar
- Bird seed Agar
- Chrom Agar

Incubation

- BOD – biological oxygen demand
- For Dimorphic fungi
  - 25°C
  - 37°C

- It takes 4-6 weeks of grow
- LPCB mount → (used to visualize)

Lactic acid
Disinfectant phenol
Cotton blue

Can be used to demonstrate hyphae as well fruiting bodies

Slide culture

- Incubated at 25°C – 27°C
- Dalmau's plate – for demonstration chlamydospores in candida

Antifungals

β - D- Glucan	Ergosterol	DNA synthesis
↓ Inhibition of synthesis ↓ Echinocandins	Ergosterol ↓ Inhibition of synthesis ↓ A- Azoles A-Allylamines P- Polyenes E- AAP	↓ Antimetabolites ↓ Flucytosine

CHROMOBLASTOMYCOSIS

- They are caused by black fungi – darkly fungi pigmented
- Sclerotic bodies are produced organisms causing chromoblastomycosis
  - F – Fonsecea pedrosoi
  - F – compacta
  - P – Phialophora verucosa
  - C – Cladosporium
  - R – Rhinocladiella aquaspersa
- Lesions caused
  - verrucose, nodular – varied

Lab diagnosis 00:02:50

- Microbiology
- Histopathological exam – cells which are called medlar cells or muriform cells

Treatment 00:03:40

- DOC – Itraeonozole – Followed by surgical interventions sometimes.

SUBCUTANEOUS MYCOSIS-SPOROTRICHOSIS

- It is subcutaneous caused by traumatic inoculation
- Distribution -found in sub-Himalayan region
- Known as Rose Gardner's disease
- Pathogenesis
  - Traumatic inoculation - splinter injury or thorn prick

↓

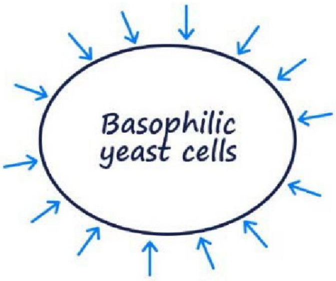
Fungi which spread by lymphatic route

- Nodule-ulcerative lesions
- Types

L	O	P	D
Lymphocutaneous type 80%	Osteoarticular ↓ Commonly seen in alcoholic	Pulmonary ↓ Inhales spores ↓ COPD	Disseminated ↓ AIDS

- Fixed cutaneous type → No lymphatic spread & only lesion

Lab diagnosis 00:05:25

Microbiology	Histopathological
1.KOH-elongated yeast cells 2.Culture - dimorphic fungi • At 25°C -mycelial form (hyphae & has conidia) is arranged in flower like pattern • At 37°C white pasty colonies classmate which turns black in color (more than 2 weeks)	Cigar shaped ↓ Asteroid bodies  Eosinophilic antigen antibody complexes splendore Hoepplii phenomenon

Serology 00:10:15

- Latex agglutination-antibodies detection
- Skin test-sporotrichin

Treatment 00:10:47

- Doc -itraconazole
- Disseminated-Amphotericin B

SUBCUTANEOUS MYCOSES-MYCETOMA

- They are chronic in source
- Most commonly by traumatic inoculation

MYCETOMA 00:00:45

- It is a chronic granulomatous disease and slowly progressive
- Causative factor-fungi & bacteria
- Pathogenesis -it is traumatic inoculation
- Triad

  1.Tumour-like appearance

  2.Multiple draining sinuses

  3.Granules-microcolonies of organism
- Types

  1.Eumycetoma
    - These are caused by fungi
    - Produce black granules
    - Seen in Rajasthan, TN,WB

  2.Actinomycetoma
    - It is caused by bacteria
    - White granules
- Lab diagnosis
  - Microbiological examination
  - Histological examination
- KOH mount

  - Eumycetoma → Hyphae with chlamydospores
  - Actinomycetoma
    - Gram staining gram positive bacilli (GPB)
    - Modified acid-fast staining →Nocardia
- Culture SDA and LJ medium
- While
  - Checking for the granules, look for
  - Texture
  - Discharge
  - Colour of granules

- In eumycetoma

Black granules caused by	White granules caused by
- M- Madurella mycetomata's and madurella grisea - E-Exophilic jeanselmi - C-curvaslaria	- F- fusarium - Aspergillus acremonium

- In actinomycetoma-white granules caused by
  - A- actinomudura madurae
  - N-Nocardia

  - S-streptomyces somallences
- Treatment
  - Eumycetoma
    - Poor prognosis
    - Almost 2yrs
    - Antifungal
    - Surgical intervention
- Actinomycetoma-Amikacin +cotrimoxazole
- Differences

Eumycetoma	**Actinomycetoma**
Swelling - single	Multiple
Discharge - serous	Purulent
Granules- black/white	White
Bone- Osteosclerosis lesions	Osteolytic lesion

SUBCUTANEOUS MYCOSES RHINOSPORIDIOSIS

Phaeohyphomycosis 00:00:12

- Caused by fungi which do not cause chromoblastomycosis
- Exists in mycelial form
- Absence of sclerotic bodies
- Agents
  - A – Alternaria
  - B – Bipolaris
  - C – Curvularia - Cladophialophora bantiana – Neurotropic → Brain abscess
  - E – Exophiala jeanselmi

Rhinosporidiosis 00:02:36

- Caused by rhinosporidium seeberi
- Friable polyps involving ear, eyes, larynx – Presentation

Pathogenesis 00:03:30

- Stagnant water

Lab diagnosis

Microbiology

- Non cultivable

Histopathological examination

Polyps

↓

Spherules

↓

Sporangium C endospores

Extra 00:06:00

- Pneumonic
  - My → Mycetoma
  - Sister → Sporotrichosis
  - Chose → Chromoblastomycosis
  - Pathology → Pharophyphomycosis
  - Robins → Rhinosporidiosis

SYSTEMIC MYCOSES HISTOPLASMOSIS

- Commonly seen when there is decrease in cell mediated immunity

Histoplasmosis 00:00:45

- Also known as darling's disease
- Granulomatous condition
- Causative agent - histoplasma capsulation
- Distribution:
  - US → Ohio valley
  - India → along Ganges River
- Pathogenesis

Spores

↓ Inhaled

Engulfed by alveolar macrophages and turn into yeast forms

↓

Enters into lymph nodes

↓

Ogans-Dissemination

Granulomas present → calcification & fibrosis

Diagnosis

Microbiology

- Culture SDA

Histopathological diagnosis

- PAS, GMS

↓

- 25°C tuberculate macroconidia & tiny smooth walled microconidia classmate
- 37°C mycelium turns into yeast

Narrow based budding of yeast

- Serology - CFT antibiotics
- Skin test - Delayed hypersensitivity reaction

  ↓

  Histoplasmin antigen
- PCR molecular diagnosis
- Treatment
  - Itraconazole is the DOC
  - Disseminated (HIV) -Amphotericin B

Types of histoplasmosis 00:10:00

1. Pulmonary
   - Most common
2. Mucocutaneous (lesions)
   - Common presentation in Indians
3. Disseminated
   - Liver, spleen
   - Common in decrease cell mediated immunity

SYSTEMIC MYCOSES-BLASTOMYCOSIS

- It is caused by a dimorphic fungus known as blastomycoses dermatitidis
- Distribution – Northern America
- It is called gilchrist's disease or Chicago disease

Pathogenesis 00:01:15

- Conidia is inhaled

  ↓

  Alveolar macrophages

  ↓

  Yeast form

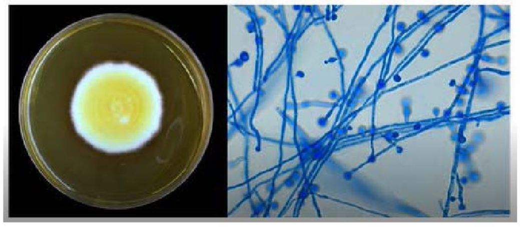
Types 00:01:45

- Pulmonary – most common
- Extra pulmonary – brain – meningitis (MC)
  - Lymph nodes
  - Liar
  - Spleen

Lab diagnosis 00:02:32

- Culture
  - 37° C ↓ Yeast mycelium ↓ Yeast from
  - 25° C ↓ Pear shaped conidia
- Histopathological PAS ↓ Blood based budding of year

  Figure of 8 appearance



Serology 00:04:26

- CFT
- ELISA

Detect antibodies

- SKIN TEST – DHT
- PCR

Treatment 00:04:58

- Itraconazole
- Amphotericin B

Doc

SYSTEMIC MYCOSES – COCCIDIOIDOMYCOSIS

- Causative agent – coccidiodes imitis – dimorphic fungi
- Distribution – arizona California
- Other names – desert rheumatism Or Valley fever

Pathogenesis 00:01:30

- Arthroconidia

↓

Inhaled into the lungs

Spherules with endospores

↓

Spherules ruptures

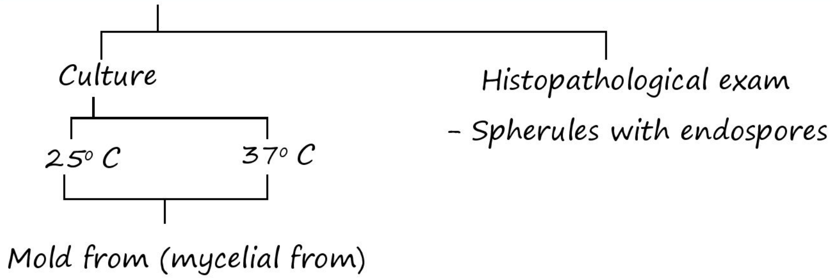
↓

Endospores release

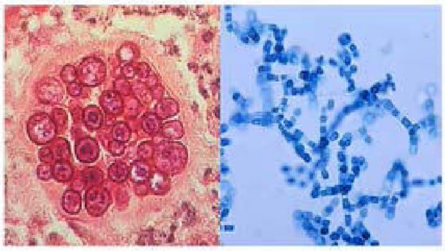
↓

More spherules

Lab diagnosis 00:02:30


Culture
25° C
37° C
Mold from (mycelial from)
Histopathological exam
- Spherules with endospores

- Fragmented hyphae with barrel shaped arthroconidia with empty cells



- Serology – antibodies antigen
- Skin test – antigen – spherule antigen
- PCR molecular diagnosis

Treatment 00:04:48

- Itraconazole
- Amphotericin B

SYSTEMIC MYCOSES PARACOCIDIODOMYCOSIS

Paracocidiodomycosis 00:00:16

- Causative organism- P. Brasiliense's
- Endemic – Bragil, Mexico
- Other name – south American blastomycosis

Pathogenesis 00:01:10

- Spores are inhaled

  ↓

- Lungs → yeast form

Type 00:01:31

1.Acute (juvenile type)

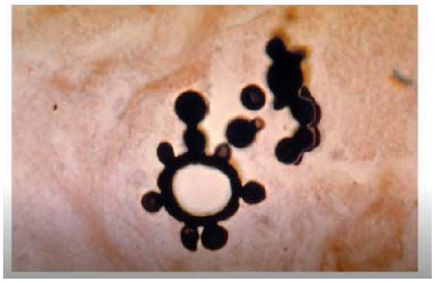
- Less common but more severe
- Seen in young
- Refractory to treatment

2.Chronic

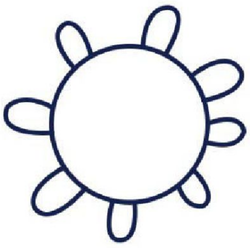
- Seen in older age group
- Less severe
- Reactivation

Lab diagnosis 00:02:45

Culture
37° C Yeast ↔ 25° C
mycelium



Histopathological
exam
↓
Yeast with brad
based multiple
buds



Plot wheel
appearance &
mickey mouse
appearance

- Serology – antibodies detection
- Skin test – DHT

Treatment 00:05:19

- Itraconazole
- Amphotericin B

SYSTEMIC MYCOSES PENICILLIOSIS

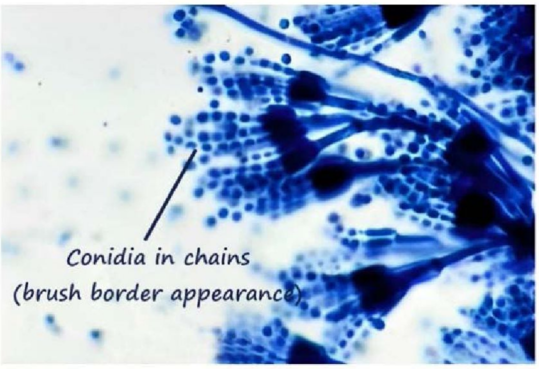
Pencilliosis 00:00:11

- Caused by pathogenic species of penicillium species
- Clinical spectrum
    - Invasive
        - Endocarditis
        - Endophthalmitis
    - Allergic
        - Bronchial asthma
        - Allergic alveolitis
    - Superficial
        - Keratitis
        - Onychomycosis
        - Otomycosis
- Penicillium species @ 25°C → mold
- Penicillium marnefeii - dimorphic
    - Molds @ 25°C
    - Yeast @ 37°C

Lab diagnosis 00:02:53

- KOH mount 40%
    - Septate hyphae
- Culture
    - ↓
    - SDA at 25°C & 37°C
    - ↓
        - Macroscopic Appearance -green cottony colonies
        - rapid growth
- LPCB mount→
    - conidiophore
    - ↓
    - Metulae
    - ↓
    - Flask shaped sterigmata
    - ↓
    - Conidia in the form of chains
    - ↓
    - Brush border appearance

- LPCB mount with penicillium



- Penicillium marneffei (Talaromyces marneffei)
- Epidemiology - south east Asian countries India - Manipur
- Pathogenesis
  - Reservoir - Bamboo rats
  - Transmission - Inhalation of conidia
- Clinical conditions

Skin lesions

- Wart like lesions

Systemic conditions

- Fever
- Breathlessness
- Cough
- Loss of weight

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Lab diagnosis 00:09:06

Culture on SDA

- 25°C

↓

Mold form

Red brick coloured pigment

- 37OC

↓

Yeast from is seen with central septations

LPCB mount

- Brush border appearance

Treatment 00:10:48

- Amphotericin B & maintenance therapy-Itraconazole

OPPURTUNISTIC MYCOSES-CANDIDIASIS

- They deal with normal commensal flora

  ↓

  Cause infection

  ↓

  When immunity is decreased (cell-mediated immunity)
- Candidiasis -candida yeast like fungi
  - C. albicans-most pathogenic
  - C. keyfr
  - C. vishwanthi
  - C. krusei
  - C. Auris

- Pre-disposing factors
  - HIV
  - Extremes of age
  - Patients on immunosuppressive drugs
  - Steroid use
  - Post transplant
  - Malignancy

Virulence factors 00:00:36

1) Phenotyping switching → candida tends to exist or change from yeast → pseudo hypha → hyphal forms
2) Candida → adhesins & invasins (adhesion to the epithelium)

↓

Hyphal form

↓

Phospholipases

↓

Infections

3) Candida lysin - peptide toxin

↓

C. albicans

4) Metabolic adaptability

- Glucose
  - Main source of carbon
  - Alternate pathway

5) zinc

- important micronutrients required by candida
- it produces zinc binding protein

↓

Scavenges for zinc in tissues

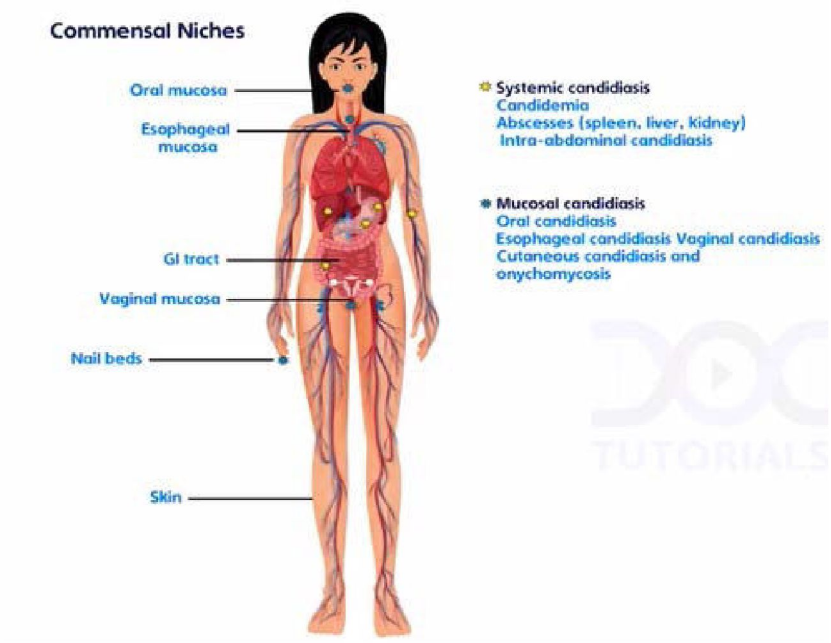
6) Goliath cells

- Spherical yeast

↓

Hyper adherent phenotype of candida

Various conditions caused 00:08:52


Commensal Niches
Oral mucosa
Esophageal mucosa
GI tract
Vaginal mucosa
Nail beds
Skin
Systemic candidiasis
Candidemia
Abscesses (spleen, liver, Kidney)
Intra-abdominal candidiasis
Mucosal candidiasis
Oral candidiasis
Esophageal candidiasis Vaginal candidiasis
Cutaneous candidiasis and
onychomycosis
TUTORIALS

- It is present as commensal Niches almost everywhere in the body and causes infection only when the immunity is decreased

Mucosal candidiasis 00:00:36

- Oral candidiasis -oral thrush
- These are white, painless patches present in the mouth

Vaginal candidiasis 00:10:53

- They present as curdy white discharge associated with pruritis
- Seen in pregnancy due to change in PH
- Denture stomatitis

Cutaneous candidiasis 00:12:17

1) Diaper rash
2) Intertrigo -pustulae
   - Lesions seen in web spaces
3) Perianal candidiasis

Candidiasis reaction 00:13:15

- Allergic reaction to candida antigen
- Candida ⎤ "Id reaction"
- Dermatophytes ⎦

Systemic candidiasis 00:14: 25

- Candidemia→ causes septicaemia
- Abscess in spleen, liver, kidney
- Intra – abdominal candidiasis
- UTI

Lab diagnosis 00:15:27

1) Gram staining
   - Gram positive budding yeast cells with pseudo hyphae
2) Culture SDA-37°C
   - Creamy white colonies on culture
   - C. albicans grows at 450C as well

Other test 00:18:02

- Dalmau's plate -species of candida can be identified

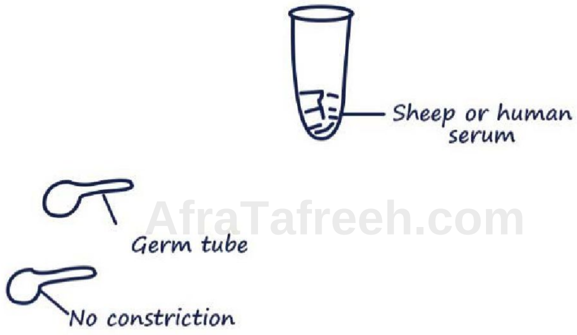
  Use ↓

- Corn meal agar→ chlamydospores are produced
- C. Krusei
- C. Glabrata

  Intrinsic resistance of antifungal drugs

- Sugar utilisation test
- Sugar assimilation test
- Germ tube test

  ↓



- C. Alibicans
- C. Dublinesis

  They produce germ tube

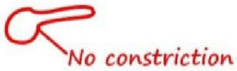
- Reynold Braun phenomenon
- CHROM agas → visual identification→ colour of various species (different species produce different colour)
- Detection of enzyme enolase
- Immunodiagnosis
  - Cell wall mannan antigen
  - Antibodies→to cell mannan

Treatment 00:23:58

1) Topical azole→ cutaneous lesion
2) Vulvovaginitis →oral fluconazole
3) Disseminated infections→ amphotericin B ± azole

Germ tube test 00:25:23

- Inoculate suspected colonies in sheep or human serum and incubate for 2hours



- Germ tube test positive
  1) Candida Albicans
  2) C. Dublinensis
- This phenomenon is known as Reynold's Braude phenomenon

OPPURTUNISTIC MYCOSES-ASPERGILLOSIS

3 important species

A. Fumigatous A. Flavus A.Niger

Risk factors 00:00:36

1.Glucocorticoid use

2.TB

3.Pneumonia

4.COPD

5.Neuropenia

Clinical spectrum 00:01:12

Pulmonary

1. ABPA
2. Allergic alveolitis
3. Bronchial asthma
4. Angio invasive pulmonary supercilious
5. Aspergilloma

Superficial/invasive

1. Endocarditis
2. Endophthalmitis
3. Invasive sinusitis
4. Maxillary fungal ball
5. Otomycosis
6. Onychomycosis
7. Mycotoxin-aflatoxin

Lab diagnosis 00:03:12

10% KOH

40% KOH

↓

Septate hyaline hyphae

Culture on SDA at 25°C

Macroscopic appearance

1.A. fumigatous-green coloured colonies

- Rapidly growing
- Powdery appearance to velvety appearance

Microscopic appearance

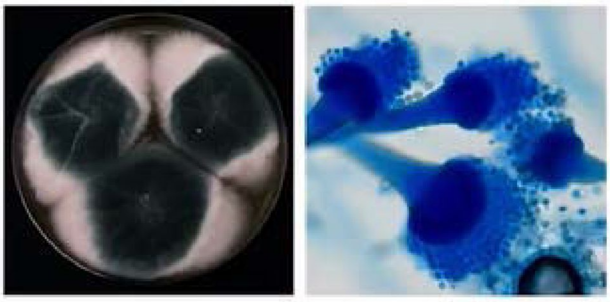
- Vesical -conical shape
- Phialides single row
- Conidia-hyaline & seen in upper half of vesicle

2. A. flavus-green-yellow in colour
   - rapid growth
   - powdery growth

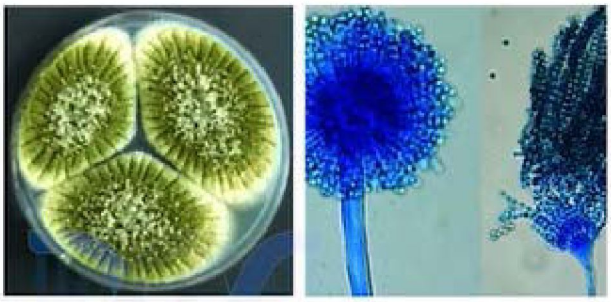
- Vesicle -globular shape
- Phialides-single or double rows
- Conidia-hyaline & seen from the entire vesicle

3. A. Niger-black -white powdery colonies
   - Rapid growth

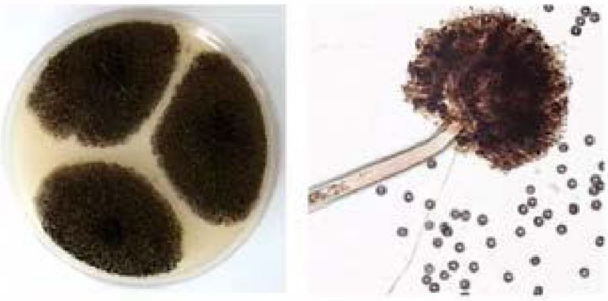
- Vesicle -globular shape
- Phialides- 2 rows
- Conidia-black coloured cruising from the entire vesicle



A. Fumigatous



A. Flavus



A. Niger

AfraTafreen.com

Other testes for detection 00:08:25

1.Antigen detection - ABPA ⎤
Asthma ⎦ Ig E elevation

2.Metabolic detection-α1, 3 glucan

Treatment 00:09:20

a)ABPA-Doc -Itraconazole

b)Invasive chronic aspergillosis-Voriconazole & Itraconazole

c)Prophylaxis-Posaconazole

OPPURUNISTIC MYCOSES- ZYGOMYCOSES

Caused by Zygomycota 00:00:46

- Aseptic hyphae
- Angio invasive

Features

Orders – Mucorales 00:01:42

a. Rhizopus

b. Rhizomucor

c. Absidia

d. Mucor

Predisposing factor 00:02:51

- Iron – requires iron to sustain life
  1. Iron therapy
  2. Diabetic ketoacidosis
  3. Neutropenia
  4. Steroid therapy

Clinical manifestation 00:03:49

a. Rhincocerebral Mucormycosis
   - Loss of vision
   - Pain in the eye
   - Ptosis
   - Seen in DKA

b. Pulmonary – 2nd most common

c. GI tract – seen in neonates causing necrotising enterocolitis

d. Other → CNS – brain

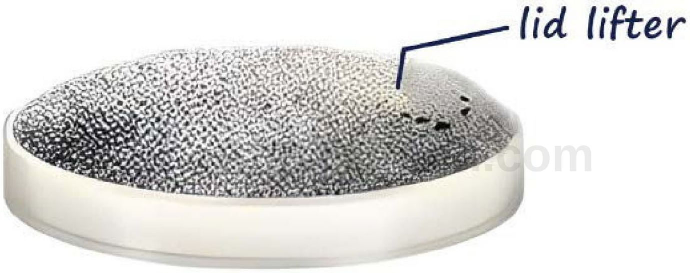
Lab diagnosis 00:05:48

a. KOH mound- Broad hyphae (aseptate) with wide angled branching

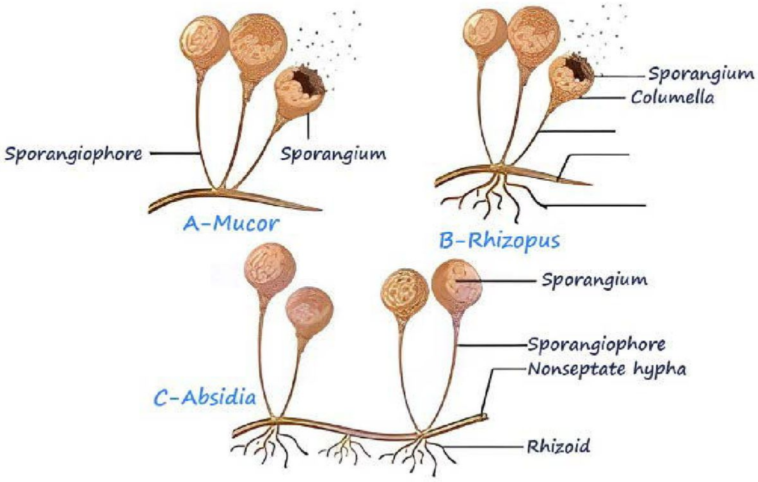
b. Culture – SDA at 25° c

↓

Wooly cotton like colonies



Rhizopus


Sporangiophore
Sporangium
A-Mucor
Sporangium
Columella
B-Rhizopus
Sporangium
Sporangiophore
Nonseptate hypha
C-Absidia
Rhizoid

LPCB mount

- CPCB mount- sporangiophore with sporangium

↓

Spores

Treatment 00:09:01

- Amphotericin B - DOC

OPPOURTUNISTIS MYCOSES PNEUMYCOCYSTIS

Pneumocystis jirovecii

- Incidence increased post HIV pandemic
- Considered to be protozoan / now grouped as fungi
- Pneumocystis carinii- infects only rat species
- Pneumocystis jirovecii- infects human species
- It exists in two forms

  Cysts   Trophozoite
- Cyst- present in environment
- Cyst & Trophozoite- seen in human body

Pathogenesis 00:02:03

- Cysts being inhaled

↓

In the lungs, it turns into trophoziod stage

↓

Causes an inflammation response

In alveoli → ↓ Plasma cells are required

Frothy exudate in the alveoli → ↓ Plasma cells pneumonia

Lab diagnosis 00:03:27

BAL, Tissue

- Histopathological examination
  ↓
  GMS- crushed ping pong ball appearance classmate seen against a green background
- It is a fungi which is not cultivable
- PCR

Treatment 00:05:16

- Doc- Cotrimoxazole- 14 days in non-HIV cases 21 days for HIV cases

↓

Prophylaxis in HIV

OPPORUNISTIS MYCOSES CRYPTOCOCCOSIS

Cryptococcus

- It is a true yeast
- Capsulated organism
- HIV pandemic has increased its incidence
- 2 spaces
  - C. Neoformans
  - C. Gattii (Immunocompetent individuals)
- Serotypes A, B, C D - Skin lesions
- Pre-disposing factors
  - HIV
  - Immunosuppressive drugs
  - Malignancy
- Virulence factors
  - Polysaccharide capsule
  - Lacteous →imp for biosynthesis of melanin
  - Melanin→ it is strong antioxidant

↓

Prevents oxidative damage g cell wall

Pathogenies 00:04:32

- Trojan Horse phenomenon
  ↓
  Infective stage- basidiospores or aerolizedspores
  ↓
  Inhaled
  ↓
  Alveolar macrophages and is phagocytosed
  - It is killed
  - immunity
    It crosses BBB like a trojan horse
- Transcytosis
  ↓
  Because of interaction of cryptococcus with CD44 of brain endothelium
- Paracellular crossing
  ↓
  Tight junctions of endothelium
  ↓
  Urease
  ↓
  Ammonia

Clinical conditions 00:07:57

- Pulmonary
- Osteolytic
- CNS
  ↓
  Meningitis
  - Fever
  - Headache
  - Cranial nerve
  - Paresis
  - Projectile
  - Vomiting
  - Memory loss
  - Sensory loss
- Skin lesion
  ↓
  Serotype

Lab diagnosis 00:09:18

a. Gram staining- Gram positive budding cells

b. Culture – SDA-white -cream pastry colonies

c. Staining

- India Ink/ Nigrosin staining → Demonstrates capsule of cryptococcus
- Modified India staining (2% Mercurichrome)

  - Alcian blue
  - Mucicarmine
  - Masson Fontana

d. special culture media

1. Bird seed agar

↓

Black colored colonies - due to the presence of melanin

e. latex agglutination test → Antigen and CSF and serum

Treatment 00:13:23

a. Without CNS involvement → Azoles and Floconazoles

b. CNS involvement - Amphotericin B

+

Azoles

POX VIRUS

- It is the largest virus
- Can be seen with light microscope
- Symmetry is complex
- Shape – Brick shaped with dumb shaped core

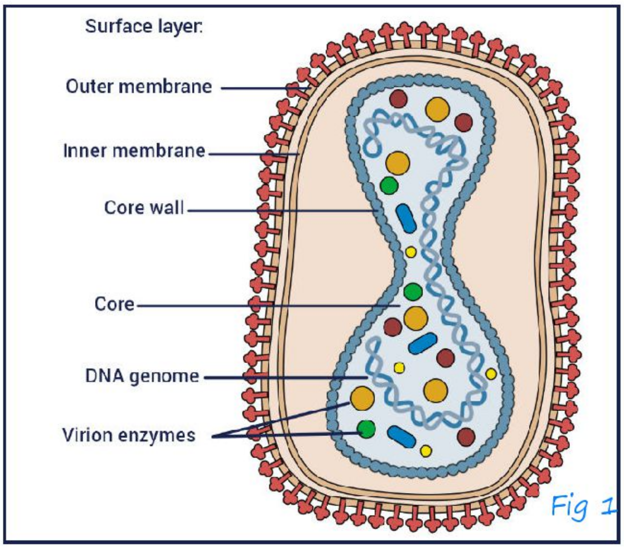

Surface layer:
Outer membrane
Inner membrane
Core wall
Core
DNA genome
Virion enzymes

Fig 1

- 4 genera of pox virus

  1) M – Mollusci pox virus

     Y- Yata pox virus
     - Yaba pox
     - Tana pox

     O – Ortho pox virus
     - Variola
     - Vaccinia
     - Cowpox
     - Buffalo pox

P – Parapox virus

- Orf pox
- Deer pox
- Seal pox
- Pseudo cowpox

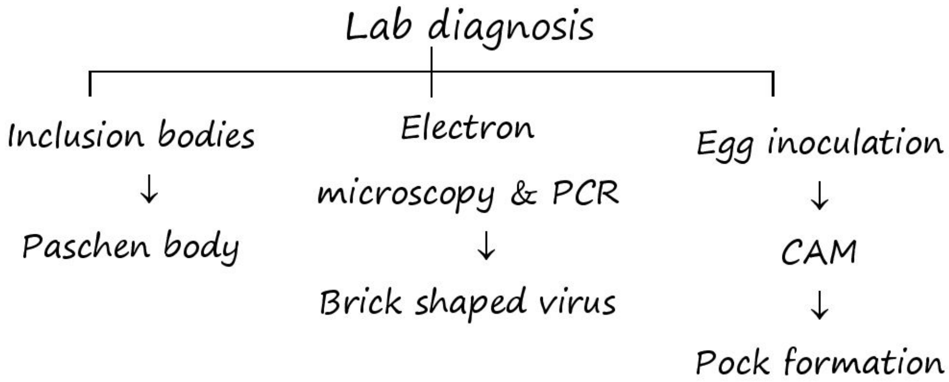
Variola

- Causative organisms for small pox
- It is the 1st eradicated disease at 1980 – WHO

Reasons

1) Affects only humans - no animal reservoir
2) Subclinical cases do not spread infection
3) There is an effective vaccine
4) No carrier stages
5) Characteristic lesion present – easy to diagnose

AmraTafreeh.com


Lab diagnosis
Inclusion bodies
↓
Paschen body
Electron microscopy & PCR
↓
Brick shaped virus
Egg inoculation
↓
CAM
↓
Pock formation

Vaccinia

- Cross reactivity against variola

Variola	Vaccinia
• CAM – small non – hemorrhagic pocks • Plaque formation in tissue culture	• Larger hemorrhagic necrotic pocks • No plaque formation

Ceiling temperatures

- Highest temperatures where the pock formation is inhibited

• 38°C	• 41°C

Chicken pox	Small pox
• Involvement of axilla & flexor surface • Lesions – pleomorphic & centripetal distribution • New crop of lesions – bout of fever is seen	• Soles, palms & extensor surface • Lesions are in the same stage ↓ Centrifugal distribution • When lesion appear, fever disappears

Variola	Vaccinia
• Paschen bodies	• Guarnieri bodies ↓ Incytoplasmic inclusion bodies

Molluscum contagiosum

- Causes pearly white lesions which are umbilicated & have dimpled center
- Seen in both children & adult

↓ ↓

Barbar Sexual route –

Swimming pool rarely

- Lesions – it does not involve soles and palms
- It is not associated with inflammation and necrosis

Lab diagnosis

- Inclusion bodies – Molluscum bodies
- PCR & electron microscopy
- It is non cultivable
- Treatment:
  - Self-limiting – 3-4 months
  - Surgical ablation
  - Cidofovir- antiviral drug

Others

1) Monkey pox –
   - Reservoir – monkeys & Rodents
   - Lesions are similar to small pox – but less serve
   - Fever and lymphadenopathy are seen
2) Pseudo cowpox
   - Milk handlers – lesions
     ↓
     Milker's nodules
3) Orf – sheep & goats
4) Cowpox & buffalo pox – Rare zoonotic disease.

HUMAN PAPILLOMAVIRUS

- It is a non-enveloped virus
- Symmetry – icosahedral
- Contains a double standard DNA
- Pathogenesis – acts on squamous epithelium & mucous

Clinical spectrum

Warts

- Common wart (veruca vulgaris)
- Plantar warts (HPV- 1)
- Flat warts (verucaplana)
- Anogenital wart
  ↓
  Condyloma acuminata
  ↓
  STD

Cervical lesions

- Cervical intraepithelial neoplasia (CIN) HPV 6,11
- Squamous cell carcinoma
  ↓
  HPV 6, 11, 16, 18, 31, 33, 45

HPV

- Recurrent laryngeal papilloma HPV 6, 11
- Malignancy HPV 16, 18

lab diagnosis

- Molecular diagnosis PCR
- Pap smear
  ↓
  Histopathological diagnosis

Treatment

- Surgical excision
- Cryotherapy
- Laser
- Topical application
  - Podophyllum
  - Imiquimod
- Malignancy – radiotherapy and chemotherapy

Cervical cancer

- It is a preventable cancer
- Vaccine
  - Subunit vaccine
    - Recombinant DNA technology
  - Quadrivalent vaccine (Gadrasil)
    6, 11, 16, 18

PARVOVIRUS

- It is the smallest virus
- It is a non-enveloped virus
- Causes erythema infectiosum
- Human infections – parvo B19

Pathogenesis

Parvovirus has an affinity for erythroid progenitor cells

↓

Mainly acts by binding to blood group P antigen which has receptors on RBC

↓

Causes lysis of RBC, inhibition of erythropoiesis

↓

Viral cytotoxicity

Clinical spectrum

Erythema infectiosum		Transient aplastic Anemia	Pure aplasia	Non immune hydrops fetalis
Children ↓ Slapped cheek appearance (Rash on disease) 5th disease	Adults ↓ Symmetrical poly arthritis	↓ Pt with Hemolytic Anemia ↓ Severe Anemia	↓ Immune compromised ↓ Severe Anemia	• Trans placental transmission • Risk – 2nd trimester

Mode of transmission

- Respiratory route
- Blood transfusion
- Transplacental transmission

Lab diagnosis

- Molecular diagnosis

  PCR – quantitative

  Genotyping – 3 genotypes are there

  For Humans – 1 is the most important genotype
- Serology ELISA – IGM and IgG
- Culture – Limited

Treatment

- No specific antiviral
- Symptomatic treatment
- Immunoglobulins

INTRODUCTION TO VIROLOGY

Human responses to virus

- Inteferons
  - Cytokines
  - They are host specific & not virus specific
- Types
  - α – fibroblasts
  - B – macrophages
  - Γ – lymphocytes
- MOA – The host cell produces "Translation inhibition proteins" → proteins synthesis
- Types

Human IFN	Pegglated IFG
• Achieved with the help of recombinant DNA technology	• Polyethylene glycol – Gives glycol – Dosage – once a week

TUTORIALS LOGIN FOR SUCCESS

What are viruses 00:03:20

- They are obligate intracellular
- Smallest unicellular organisms
- Either DNA or RNA, never both
- No cellular organization

- Do not have enzymes required for protein synthesis & DNA synthesis
- Do not grow on artificial media
- No role of antibiotics

Structure & symmetry 00:05:20

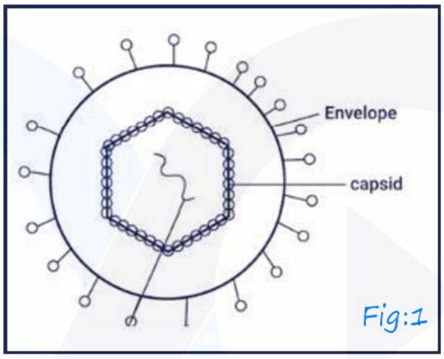


Fig:1

- They contain nucleic acid DNA or RNA

  ↓

  This is enclosed by capsid (protein coat)

  ↓

  Contains subunits → capsomers

Capsomers

↓

Symmetry

Icosahedral | Helical | Complex- Pox virus

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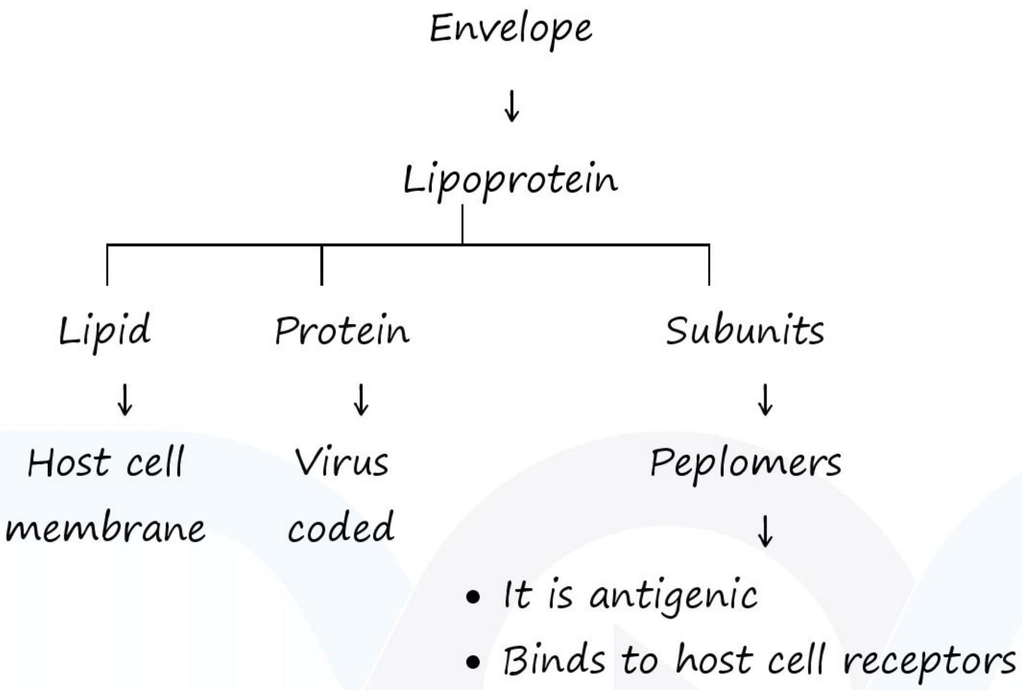
Envelope 00:07:53


Envelope
↓
Lipoprotein
Lipid
↓
Host cell membrane
Protein
↓
Virus coded
Subunits
↓
Peplomers
↓
• It is antigenic
• Binds to host cell receptors

Non – enveloped viruses 00:09:15

- DNA viruses
  - P – parvovirus
  - A – adenovirus
  - P – Papovavirus
- RNA viruses
  - Picorna
  - Hepatitis A & E

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Size 00:10:20

- Parvo → smallest virus
- Pox → largest virus
- Measured by

  1.Electron microscope

2.Ultra centrifugation

3.Filter – membrane with different pore size

Shape 00:11:32

1.Brick shaped – pox virus

2.Bullet shaped – Rhabdo virus

3.Filamentous – Ebola virus

4.Petal of lower – corona virus

Virus replication 00:13:00

- Steps

    Attachment
    ↓
    Penetration
    ↓
    Uncoating
    ↓
    Biosynthesis
    ↓
    Assembly

- Variation in symmetry & structure

Virioids	Prions	Virusoids
• Contains SS RNA without capsid	• Infectious protein with nucleic acid	• Depends on other viruses for replication

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Abdominal replication

1.Incomplete viruses → capsid without NA

↓

Influenza virus →

- low infectivity
- ↑ HA titres
- Von Magnus phenomenon

2.Pseudo virion → Capsid enclosed host nucleic acid

3.Abortive infection → Virus enters non – permissive host cells

4.Defective viruses → Viruses which depend on other viruses to complete replication. e.g., Hepatitis D

5. defective interfering viruses

Viral genetic modifications 00:18:05

Mutation	**Viral genetic exchange**	**Viral products**
Reassortment ↓ Influenza ↓ 2 strains infect host cells ↓ Exchange in nucleic genome	Similar viruses ↓ Exchange of viral genome	Genetic modifications ↓ Marker rescue

Viral product exchange

Viral product

- Phenotype mixing
- Genotype mixing
- Pseudo type formation
- Viral interference

Viral interference
↓
2 viruses (A+B)
↓
Host cells
↓
A inhibits B

- Compete for component of replication
- Binding of host cell receptors
- Destroys host cell receptors

OPV – 3 serotypes
↓
Vaccine failure

Morphological changes 00:22:38

Morphological changes

- Damages host chromosome
- Inclusion bodies

Damages host chromosome

- Chromatin rings around nuclear membrane of host cells
  e.g., HSV and adenovirus

Inclusion bodies 00:23:55

1.Intracytoplasmic inclusion bodies

- Negri bodies
- Paschen bodies
- Guarnieri bodies
- Bollinger bodies
- Molluscum bodies

2.Intracellular inclusion bodies

- Cowdry type A
  - Torres body
  - Lipschultz
- Cowdry type B
  - Polio virus
  - Adenovirus

3.Intracytoplasmic & intranuclear inclusion bodies

- CMV – owl's eye

Lab diagnosis 00:26:56

Lab diagnosis

- Electron microscope
- Inclusion bodies can be seen with light microscope
- Antigen Detection
- Antibody Detection
- Molecular methods PCR
- Virus isolation
  - Embryonated egg
  - Tissue culture
  - Animal inoculation

Isolation

1.Animal inoculation

- Suckling mice → done in cocksackie virus – intracerebral

  A → flaccid paralysis → AFB

  B → spastic paralysis

2.Embryonated egg

TUTORIALS LOGIN FOR SUCCESS

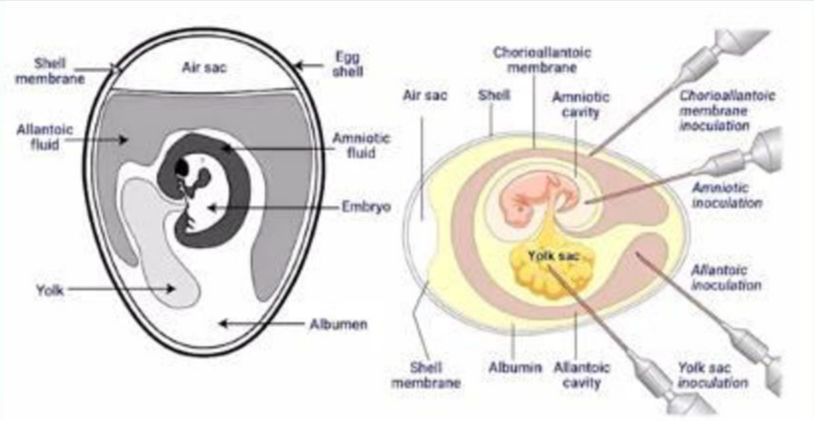
Shell membrane
Air sac
Egg shell
Allantoic fluid
Amniotic fluid
Embryo
Yolk
Albumen
Chorioallantoic membrane
Air sac
Shell
Amniotic cavity
Chorioallantoic membrane inoculation
Amniotic inoculation
Yolk sac
Allantoic inoculation
Shell membrane
Albumin
Allantoic cavity
Yolk sac inoculation

Fig:2

- Yolk sac inoculation

  a. Chlamydia

  b. Arboviruses

  c. Rickettsia
- Allantoic inoculation – used for vaccine production

  – 17 D – flurry strain of rabies
- Amniotic inoculation – influenza virus
- Chorioallantois membrane inoculation

  a) Variola

  b) Vaccine – produces necrotic, haemorrhage pock

  c) HSV – HSV – 2 bigger pocks than HSV – 1

3. Tissue culture

1) Primary cell line
- Normal cell
- 5 -10 division
- Diploid karyosome

e.g., monkey kidney cell line

2) 2° cell line
- Normal cell
- 10 -50 division
- Diploid karyosome

3) Continuous cell line
- Cancer cell
- Infinite
- Haploid karyosome

e.g.,
Hela virus
Hep 2
Vero virus

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Detection changes in tissue culture

Cytopathic changes

- Degeneration of entire cell line ↓ Enterovirus
- Ballooning ↓ HSV
- Vacuolation ↓ SV 40

Viral interferences 00:34:50

- Detect a non-cytopathic virus

Changes in tissue culture 00:36:22

Changes

↓

Cytopathic effect → morphological changes

- Degeneration of entire cell line ↓ Enterovirus
- Ballooning of cells ↓ HSV
- Syncytium formation ↓ RSV HSV
- Granular ↓ Grape – like ↓ Adenovirus

TUTORIALS LOGIN FOR SUCCESS

HERPES SIMPLEX VIRUS – PART -1

Herpes virus 00:10:00

Subfamily	latency	species
Alpha	Neurons	Herpes virus 1 Herpes virus 2 Herpes virus 3
Beta	Kidney T cells	Herpes virus 5 Herpes virus 6 Herpes virus 7
Gamma	B cells	Herpes virus 4 Herpes virus 8

- Herpes 3 → Varicella zoster
- Herpes 5 → CMV
- Herpes 4 → EBV
- Herpes 8 → Kaposi sarcoma causing herpes

TUTORIALS
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Herpes simplex virus 00:02:11

Properties	HSV -1	HSV -2
Mode of transmission	– Direct contact	– Sexual or vertical
Latency	– Trigeminal ganglia	– Sacral ganglia
Age	– Children	– Adults
Manifestation	– Lesions above the waist – Encephalitis – Ocular lesions	– Lesions below the waist – Neonatal herpes
CAM	– Smaller pocks	– Larger pocks

Manifestation

- MC – Orofacial
- MC site – buccal mucosa
- Condition
  - Herpes labialis – painful vesicular lesions around the lips
  - Commonly associated with HSV – 1
  - Others
    - Tonsillitis
    - Pharyngitis
    - Gingivostomatitis

Ocular lesions – HSV 1 > HSV 2

1. Conjunctivitis
2. Dendritic ulcer
3. Corneal opacity

Cutaneous manifestation – HSV 1 > HSV 2

- Herpetic whitlow
- Herpes gladiatorum
  ↓
  wrestlers
- Eczema herpeticum
- Erythema multiform

CNS manifestation HSV 1 > HSV 2

1. Encephalitis → HSV – Acute sporadic
   ↓
   MC viral encephalitis
2. Mollaret's Meningitis

TUTORIALS
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↓

Recurrent lymphatic meningitis

3.GBS

↓

Transverse myelitis

Genital lesions – HSV 2 > HSV 1

- B/L Painful lesions with fever and lymphadenopathy

Neonatal herpes – HSV 2 > HSV 1

- Asymptomatic
- Spread during delivery
- Clinical manifestation
  - Encephalitis
  - Lesions around the mouth
  - Disseminated disease

Lab diagnosis 00:08:55

1 Inclusion bodies

- Lipschultz body
- Multinucleated giant cells

2 Virus isolation

- Can be done by tissue culture
- Ballooning of cells can be seen
- Shell vial culture – 1-2 days

3 PCR

4 Antigen detection by IF

5 Antibody detection

TUTORIALS LOGIN FOR SUCCESS

HERPES SIMPLEX VIRUS – PART 2

Varicella zoster 00:00:15

1. Chicken pox
2. Zoster or shingles

1. Chicken pox

- A case of chicken pox → spread
- Disease of childhood
- Secondary attack rate – 70-90%
- Manifestation
  - Centripetal
  - B/L
  - Painful
  - Multiple crop – pleomorphic lesion
  - Fever with every new crop
- MC complication – 2° bacterial infection
- Most dreaded complication – varicella pneumonia
- CNS
  - Encephalitis
  - Meningitis

Chicken pox in pregnancy

- Mother
  - ↑ Risk of pneumonia
- Baby
  - 1st half ↓ asymptomatic
  - 2nd half ↓
    - Congenital varicella syndrome
    - Triad
      - Lesion
      - Hypoplasia of limbs
      - chorioretinitis

TUTORIALS LOGIN FOR SUCCESS

Delivery

- > 5 days before delivery
- Asymptomatic
- Protection due to maternal antibodies

- Less than 5 days and up to 2 days after delivery
- Neonatal varicella

Varicella zoster 00:04:50

- Reactivation of varicella virus
- Latent – present in ophthalmic branch of trigeminal nerve
- Appears in old age or immunocompromised
- Clinical manifestations

1.Post – herpetic neuralgia

- Pain at local site

2.Ramsay hunt syndrome

- Facial nerve palsy

+

Lesion in tympanic membrane, external auditory meatus

- Geniculate ganglion of facial nerve

3.Zoster ophthalmicus

- Lesions seen ganglion of facial nerve

TUTORIALS LOGIN FOR SUCCESS

Lab diagnosis 00:06:50

1.Inclusion bodies

- Done of Tzanck smear
- Multinucleate giant cells

2.Virus isolation

↓

Tissue culture

↓

Ballooning of cell line

3.PCR

4.Antigen detection by IF

5.Antibody detection

Treatment 00:08:06

- Acyclovir
- Vaccination – live attenuated vaccine – Oka strain

  ↓

  After 1 year of age → 2 doses
- Immunoglobins → less than 72 hours

TUTORIALS
LOGIN FOR SUCCESS

HERPEX SIMPLEX VIRUS – PART – 3

Cytomegalovirus (CMV) 00:00:10

- Largest of Herpesviridae family
- Clinical manifestation
  - Congenital
    - MC intrauterine condition to cause congenital defects
      - Microcephaly
      - Intracerebral calcification
      - Petechiae
      - Hepatosplenomegaly
      - Jaundice
      - chorioretinitis
  - Adult
    - Immune competent ↓
      - Mononucleosis like syndrome
      - Post – transfusion
      - Paul Bunnel test negative ↓ No heterophile antibodies
    - Immune compromised
      - HIV
        - CD 4 count less than 50/ML
        - Can cause
          - Chorioretinitis
          - Dementia
          - gastroenteritis
      - Transplant

Transplant

- 1–4 months post-transplant
- Bone marrow transplant
- Solid organ transplant – febrile neutropenia
- Lung – Obliterative bronchiolitis
- Heart – Graft atherosclerosis
- Renal – Rejection

Lab diagnosis 00:04:18

1.Inclusion body

- Owl's eye appearance

2.Virus isolation by tissue culture

TUTORIALS LOGIN FOR SUCCESS

↓

- Multinucleate giant cells
- Increase in size of host cells

3.Antigen CMV specific PP65 antigen

4.Antibody detection

- ELISA
- CLIA

Treatment 00:05:43

- Ganciclovir
- Foscarnet in ganciclovir resistance
- Inclusion body → owl's eye appearance

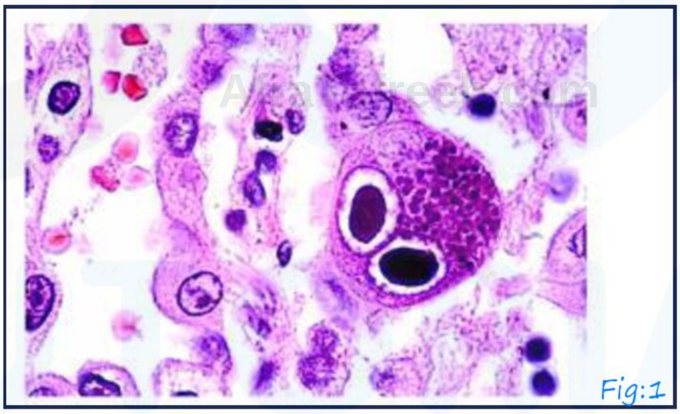

Fig:1

LOGIN FOR SUCCESS

HERPES SIMPLE VIRUS – PART – 4

Epstein Barr virus 00:00:10

- Herpes virus 7
- Antigen
  - Early antigen (EA)
  - Late antigen (LA)
  - Latent antigen
    - EBNA
      ↓
      Nuclear antigen
    - LMP
      ↓
      - Membrane protein
      - LMP – 1 – viral oncogene

Clinical manifestations 00:01:18

a. Infectious mononucleosis

- Fever
- Pharyngitis
- Cervical lymphadenopathy
- Atypical lymphocytosis

b. Carcinoma associated with EBV

- Burkitt's lymphoma
  ↓
  Children
  ↓
  Tumor of jaw mutation – t (8, 14)
- Nasopharyngeal carcinoma
  – Nitrosamine

TUTORIALS
LOGIN FOR SUCCESS

↓

Chinese

- Hodgkin's lymphoma mixed cellularity type
- NHL

➢ Others - Hairy cell leukoplakia

Lab diagnosis 00:04:18

- Antibody detection
- PCR → sensitive and specific
- Virus isolation – not done
  - Labour intensive
  - Time taking

Antibody detection

Heterophile

- Paul test Bunnell – tube agglutination test
- Inactivated serum + 1% sheep RBC

↓

Incubate @ 37° c for 48 hours

↓

Titer > 256 - significant

EBV specific antibodies

EBNA
EA
LA

EA ↓ Nasopharyngeal carcinoma

- Monospot test – slide agglutination test
  - Horse RBC instead of sheep RBC
- Differential adsorption test

Serum + ox RBC's

↓

Paul Bunnel test

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TUTORIALS
LOGIN FOR SUCCESS

HERPES VIRUS – PART-5

- Herpes
- Other virus

Herpes virus- 6	Herpes virus -7	Herpes virus – 8
• 6th disease ↓ Exanthum Subitum Or Roseola infantum • It binds to CD34 receptor	• Affects T cells • Children	• Kaposi sarcoma associated herpes virus • Affect T cells • Kaposi sarcoma • Primary effusion lymphoma • Mastleman's disease

ADENO VIRUS

Adeno virus 00:00:10

- Double stranded (ds) DNA
- Non-enveloped
- Space vehicle shaped – icosahedral symmetry

  ↓

  Fiber protein is projected from each vertex
- Most important serotype is between 1 – 7

Clinical spectrum 00:01:18

1.Respiratory

- URTI

  (children)

  Serotype 1,2,3,5
- Pneumonia

  3,7,21
- Acute respiratory disease

2.Ocular

- Children

  Swimming conjunctivitis

  3,7
- Adult

  Shipyard eye

  8,9,37

3.Others

- Infantile gastroenteritis
  40,41
- Acute cystitis
  2,11
- Transplant
  - Colitis
  - Cystitis
  - 34,35

Lab diagnosis 00:04:41

Tissue culture	Antigen detection in clinical species	Antibody detection	PCR
• Hela Hep -2 KB cell line ↓ Cytopathic effect ↓ - Grape like clusters of cells - Detect antigen - IF	• IF	• CFT • ELISA • HAI	• Specific for identification

PARAMYXOVIRUS (MEASLES VIRUS)

Measles 00:00:10

- Common childhood disease
- Highly contagious → 2° attack rate →90%
- Source – case of the disease
- Reservoir – Humans
- Route – respiratory route

Clinical manifestations 00:01:28

- Incubation period – 10 days
- 1st symptom – fever – 10th day of infection

↓

Koplik spots → Bluish spots with erythema

↓ ↓

2nd lower molar

Rash → 14th day of infection

Maculopapular rash

→ 1st – Ear

↓

Face

↓

Arms

↓

Trunk

Complications 00:03:36

- 2° bacterial infections (most common) → otitis media, pneumonia
- Complications because of measles
  - Giant cell pneumonitis
  - Croup
- CNS → post measles encephalomyelitis (SSPE)

Lab diagnosis 00:05:04

1. Cell line culture

   ↓

   Giant cells

   ↓

   Warthin – Finkeldey cells
2. Antigen detection
3. Antibody detection
4. RT - PCR → RNA

Vaccination 00:06:04

- Edmonston strain

  ↓

  Schwartz strain (used worldwide now)
- Edmonston – Zagreb stain
- After 9 months (9-12 months)

  ↓

  2nd dose (24 months)

Prevention 00:07:21

- MMR vaccine
- Tetravalent vaccine – MMR-V vaccine
- One attack gives lifelong protection

PARAMYXOVIRUS (MUMPS VIRUS)

Mumps virus 00:00:10

- Most common cause of enlargement of parotid gland in children
- IP – 19 days
- Humans are the only reservoir
- Communicabitlity – one week before to one week after the symptom's onset
- SAR → 86%
- It gives protection for life→ after attack
- Most common manifestation – B/L parotitis
- Most common complication – epididymo – orchitis

↓

Unilateral sterility is uncommon

- Others → complications

a. Oophoritis

b. Pancreatitis

c. Atypical mumps

  - Aseptic meningitis
  - No B/L parotitis

d. Aseptic meningitis

Vaccination 00:03:19

- Jeryl- lynn stain used
- Trivalent – MMR
- Tetravalent – MMR – V
- Monovalent – mumps

PARAMYXOVIRUS – RSV AND PARAINFLUENZA VIRUS

Respiratory syncitial virus (RSV) 00:00:10

- Most common cause of respiratory illness in less the 1-year children
  - Bronchiolitis
  - Pneumonia
- Influenza like symptoms in adults
- Exacerbation of asthama, COPD

Lab diagnosis

- Cell line → HCLA & Hep 2

↓

Syncitium formation (multinucleated giant cell)

Treatmemt

- DOC – Ribavarin (Aerozolised form)

Parainfluenza virus 00:03:33

Clinical symptoms

- Common cold syndrome – Rhinitis & pharyngitis
- Croup type 1 & type 2
- Pneumonia & Bronchiolitis type 3

Complication

- Otitis media

RUBELLA VIRUS

Rubella virus 00:00:10

- Also knowm as German measles
- Measles (Rubeola virus)
- Togaviridae
- IP – 14 days
- No carrier – source -case of Rubella
- Once infected → lifelong immunity

Clinical spectrum 00:01:20

In adults

- Subclinical cases – 50%
- Rash – day 1

  ↓

  Lymphadenopathy (post auricular)

  ↓

  Forschheimer spots → soft palate uvula

In children

- Congenital rubella syndrome

  a. Occular

    - Salt & pepper retinopathy (most common)
    - Cataract

  b. Ear

    - Deafnesss (most common)

c. CVS

  - PDA (most common)
  - Pulmonary stenosis
  - VSD

- Transient changes
  - Hepatosplenomegaly
  - Petechiae
  - Jaundice

Bluebeberry muffin syndrome

Vaccination 00:04:40

- Reproductive group
- C/I in pregnancy
- It is teratogenic → no pregnancy should be planned up to 28 days post vaccination

Lab diagnosis 00:05:23

A. Cell line

- Viral interference detected by echovirus

B.Antibody detection

- IgM in neonate – Rubella infection
- IgM → maternal antibodies

Or

Infection

↓

Detected after 9 months

RABIES VIRUS

Rhabdoviridae 00:00:10

Rhabdoviridae

Lyssavirus

↓

Rabies like illness

Vesiculovirus

- Morphology – Bullet shaped

Envelope

Nucleocapsid

- Lipid bilayer
- Lined matrix protien
- Glycoprotein G (seen in the form of spikes)

Present in subunit vaccine

↓

Helps in neutralising antibodies

Neuroinvasiveness & pathogenecity

↓

Binds to Ach receptor in neural tissues

Street virus	Fixed virus
• Freshly isolated in lab • Resemble the wild virus • Long incubation periods • Produces inclusion bodies	• Propagating by brain – brain passage in rabbits • Multiplies rapidly • Does not produce inclusion bodies • Properties – few are lost • Isolated from neural tissue • Not seen in salivary gland • Used for vaccination

Pathogenesis 00:04:05

- Most common is by the bite of dog
- Others are by fruit bats, buffaloes, fox, goats
- Other modes are by lick of mucosa, corneal transplant & Aerosol route
- Virus replicates at the site of inoculation

  ↓

  Transports centripetally through peripheral nerves after binding at NMJ (Ach receptor)

  ↓

  Dorsal route ganglia of spinal cord & CNS

  ↓

  CNS → cerebellum & hippocampus

  ↓

Centrifugally its spread through sensory nerves to cornea & salivary gland

↓

Virus shed in saliva

- Pathological finding is presence of Negri bodies

↓

Intracytoplasmic eosinophilic inclusion bodies

Clinical spectrum 00:07:25

A. Prodromal stage

- Fever
- Malaise
- Photophobia
- Pain at the site of bite

B.I – Encephalitic stage (80%)

↓

  - Lucid interval
  - Hydrophobia
  - Hyperexcitability
  - Autonomic dysfunction
  - Increase salivation

II Flaccid stage (20%)

  - Flaccid paralysis

III Coma & death

Lab diagnosis 00:09:41

A. Antigen detection

- Done by detecting nucleoprotein by IF
- Specimen – hair follicle from nape of the neck, corneal smear

B. Antibody demonstration

- Serum & CSF

↓	↓
Late & can also be seen in vaccinated individuals	• Specific • Seen only in a person affected by rabies

- Methods
  - CFT
  - ELISA
  - HAI

C. Microbiological

I. Animal inoculation	II. cell lines
↓	↓
• Intracerebral inoculation in sucking mice ↓ – Encephalitis – Depth • Brain biopsy – Negri bodies	• BHK cell line • Mouse neuroblastoma cell line

D. Pathological findings

- Negri bodies → post morterm finding
- H & E or sellar strain
- Cerebellum & hippocampus

Treatment 00:13:58

- Symptomatic
- Sedatives
- Anti-anxiolytics

Prevention 00:14:20

A. Local

- Wash wound with soap & water
- Prophylactic antibiotics
- T.T given
- Do not suture the wound site
- Debridement

B.Administration of immunoglobulins

↓

At the site of bite

↓

Neutralize the virus

- 2 types
  - Equine
  - Human
    - No side – effects

A. Neural vaccine

- From neural tissue of infected animals by fixed virus
- Side effects
  - Encephalitogenic
  - Less immunogenic
- SBI
  - S – semple vaccine inactivated by phenol
  - B – BPL – inactivated by BP2
  - I – Infant mouse vaccine

B.Non neural vaccine

I – Egg based vaccine

↓

Derived from allantoic cavity

PDEV

↓

Duck layer

allantoic cavity

Live attenuated chick embryo vaccine

II – Recombinant vaccine

↓

Vaccinia virus & used for glycoprotein gene

III– Cell culture vaccine

- Most commonly used
- Most advised

- Cell culture vaccine
  - Highly recommended
  - Non encephalitogenic
  - Highly immunogenic

PCEC

- Chick fibroblast cell line

PVC

- Vero cell line

HDC

- WI – 38 (human embryonic lung fibroblast cell

Post – exposure prophylaxis 00:21:23

- ➢ IM or Essen regimen
  - Given in deltoid region
  - 5 doses (1-1-1-1-1) → 0,3,7,21,28 day

- ➢ ID
  - 0,3,7,21 or 28 days (2-2-2-0-2)

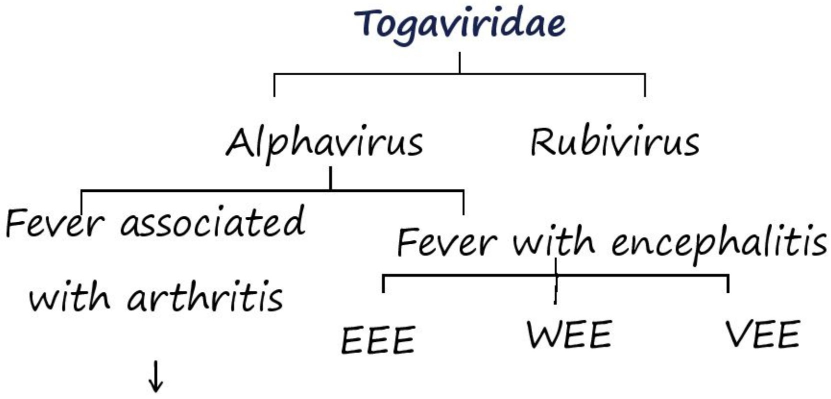
↓

1st day of vaccination

ARBOVIRUSES

Arboviruses

- RNA viruses
- They are transmitted by arthropods
- Commonly associated with 5 families


Togaviridae
Alphavirus
Rubivirus
Fever associated with arthritis
Fever with encephalitis
EEE
WEE
VEE
↓

- Chickengunya
- Onyongnyong
- Mayoro virus
- Ross river virus
- Semelik forest virus

Chikungunya

- Fever with arthritis
- Endemic outbreak → 2005
- 2 types

  a. Urban cycle
    - Reservoir – main
    - Vector – aedes mosquito

b. Sylvatic cycle

- Reservoir – primates
- Vector – forest related aedes mosquito

- Clinical features
  - Fever
  - Joint pains
    - Polyarticular migratory – mostly involves small joints
    - Inflammation
    - Takes years to recover

- Lab diagnosis

Virus isolation	**Antibody detection**	**PCR**
↓ Done by mosquito cell line	– IGM antibodies acute – IgG recovery or past infection – 4-fold in titre used	– Detect the RNA – sensitive and specific

- Treatment – Symptomatic
- ONN virus
  - Similar symptomatic
  - Transmitted by anopheles' mosquitoes
  - Mayaro virus ⎤ Aedes mosquito
  - Ross river virus ⎦
  - Similik forest virus
  - Sindibis – culex mosquito

- Chickengunya outbreak in 2005 due to
  - Mutation – Alanine in 266 of E1 glycoprotein from valanine
  - Vector – A.Albopictus

Fever with encephalitis 00:11:35

EEE	WEE	VEE
↓	↓	↓
Aedes mosquito	Culex mosquito	By both aedes &

Flaviviridae 00:13:31

Mosquito – transmitted flaviviruses		Tick transmitted flaviviruses	
Encephalitis group ↓	Hemorrhagic fever group	Encephalitis group	Hemorrhagic group
• Japanese B encephalitis • West Nile • Murray valley encephalitis virus • ST. louis encephalitis virus • Rocio encephalitis virus	• Dengue	• TBE viruses • Powarsan encephalitis virus • Looping ill virus	• KFD

Japanese B encephalitis 00:15:55

- MCC of viral encephalitis
- Seen mainly in children
- 20-40% mortality rate
- Vector – culex mosquito
- Hosts
  - man → main
  - pigs → JE virus multiplies → amplifier host
  - bird
- endemicity – south east Asian countries
- UP, WE & Assam
- Seasonality – rainy season

Clinical features

- IP – 5-15 days
- Subclinical – iceberg phenomenon
- Clinical
  - a. Prodromal stage
  - b. Fever with encephalitis
  - c. Sequelae (20-40%)

Vaccination

1. Live attenuated vaccine – SA 14-14-2 vaccine
   - Cell line derived vaccine
   - Primary hamster kidney used
   - Subcutaneous route – 1 dose
   - Booster after 1 year

2. Inactivated cell line derived vaccine (Beijing p3 strain)

3. Inactivated vaccine
   - Mouse brain derived & formalin inactivated
   - Called as Beijing & Nakayama strain

Haemorrhagic fever group 00:22:08

Dengue

- MC arbovirus in India
- Serotypes – DEN 1 to DEN 4 (DEN 2 – most dangerous)
- Vector – Aedes aegypti & aedes albopictus
- Reservoir – man
- Pathogenesis – antibody mediated

Neutralizing antibodies	Non-neutralizing antibodies
• Produced against infected serotypes ↓ Lifelong immunity • Produced against other serotypes ↓ Short while	• Another serotype but not the infecting serotype ↓ Inhibits bystander B-cell activation ↓ Antibody dependent enhancement ADE

Clinical spectrum

a. Dengue fever
b. Dengue haemorrhagic fever
c. Dengue shock syndrome

Lab diagnosis

1. Antigen detection	2. Antibody	3. Virus isolation
➢ NS 1 antigen • Positive from day 1-18 • 2 methods - ICT - ELISA	➢ 1^{o} infection • Igm positive – day 5 • IgG – 2nd week 14-21 days • 2^{o} infection Rapid increase in IgG - MCA ELISA – MC - CFT - ICT - Rapid detection	➢ Mosquito cell line ↓ Molecular method PCR ↓ Most sensitive & specific

Treatment

- Symptomatic
- Correct electrolyte imbalance
- Platelet transfusion

Yellow fever 00:29:17

- Not found in India
- Vector- aedes aegypti
- Reservoir – man
- It is associate with jaundice, haemorrhage & platelet dysfunction
- Reasons for absence of yellow fever in India

  a. Dengue is endemic

  ↓

  Dengue antibodies are protective against yellow fever

  b. Strong vigilance → aedes aegypti index (less than 1)/ Breteau index

  c. Quarantine

  ↓

  Unvaccinated people are quarantined for longest incubation period

Clinical feature

- Jaundice
- Fever
- Malaise
- Body pains
- Hepatitis → intranuclear inclusion bodies – Torres bodies

Vaccination

- 17D vaccine → Allantoic cavity of chick embryo

  ↓

  - C/I → egg allergy
  - S/C route
  - Single dose

    ↓

    7 days – 35 years
  - Vaccination certification

    ↓

    10 days of vaccination

    ↓

    Valid for 10 days

KFD (Keyasanur forest disease) 00:35:10

- Vector – hard tick
- Host – man, rodents, squirrels, monkeys- amplifier host
- Endemicity – Karnataka
- Clinical manifestation/ spectrum
  - 1st stage – fever with haemorrhagic symptoms
  - 2nd stage – meningoencephalitis
- Vaccination → killed KFD vaccine

  ↓

  Formalin inactivated chick embryo vaccine
  - given in endemic area

Remaining arboviruses 00:37:57

3 families

- Bunyaviridae
  - Bunyavirus
  - Nairo virus ↓
    - Crimean Congo hemorrhagic fever
    - Ganjam virus
  - Phlebovirus
- Reoviridae
- Rhabdoviridae ↓ Chandipura virus ↓
  - Sandfly
  - Children

MYXOVIRUSES (ORTHOMYXOVIRUSES)

Orthomyxoviruses 00:00:10

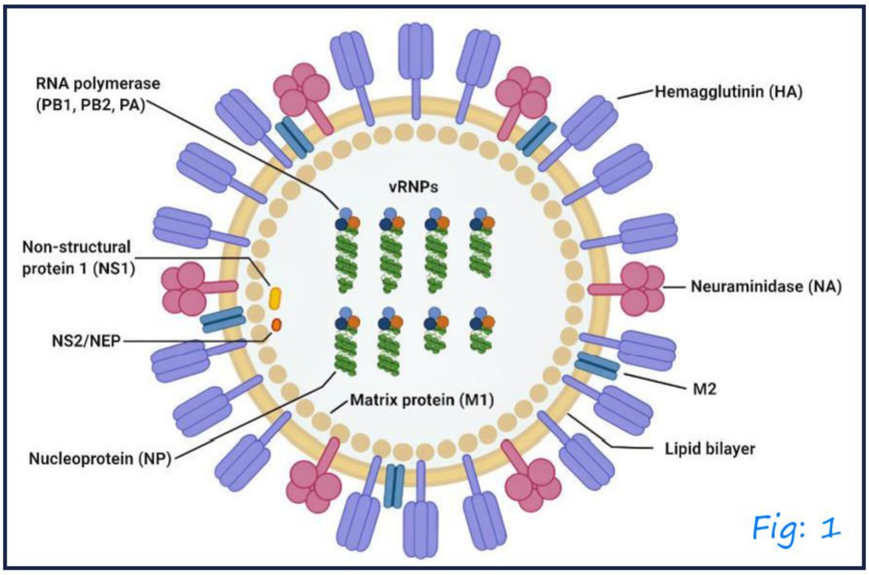

RNA polymerase
(PB1, PB2, PA)
Hemagglutinin (HA)
vRNPs
Non-structural
protein 1 (NS1)
Neuraminidase (NA)
NS2/NEP
M2
Matrix protein (M1)
Lipid bilayer
Nucleoprotein (NP)

Fig: 1

- Shape – spherical
- Symmetry – Helical
- Nucleic acid – SSRNA (segmental) → 8 pieces
- Replication – nucleus
- 2 antigens → 2 glycoproteins

Haemagglutinin (HA)	**Neuroaminidase(NA)**
• Hemagglutination • Bind micin or sialic receptors ↓ Clumping of RBC	Reversal of HA ↓ Elution

- Typing – 4 genera

  a)Type A → most common in causing epidemics

  b)Type B → also causes epidemics

  c)Type C → not in circulation

  d) Type D → affects cattle

Sub types

- Classified based on HA & NA
- HA → H (1-18)
- NA → N (1-11)

Antigenic shift	Antigenic drift
• Caused by Type A • Genetic reassortment • 10 – 20 years • Pandemic & epidemics	• Type B & ration A • Point mutation • 2 – 3 years • Epidemics

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Lab diagnosis 00:04:50

- Lab diagnosis

↓

Specimen & transport → Nasopharyngeal swabs

↓

Transported in VTM (virus transport medium)

At 4°C & later stored for 4 days & later has to be stored at -70°C

i) Virus isolation → Monkey kidney cell line ii)Egg inoculation

Amniotic	**Allantoic**
• Type A, B, C • A – agglutinates pig RBC • C – foot RBC'S • B – both	• Type A

iii) Molecular methods

- RT – PCR
- Realtime RT-PCR – Quantitative estimation of viral load

iv) Antigen detection - IF → nasopharyngeal cells

v)Antibody detection

- 4-fold rise in titer
- HAI
- Neutralization test
- ELISA

Vaccines 00:07:16

---

1) Injectable vaccines

a)Inactivated influenza vaccine

- Inactivated by formalin BPL
- Allantoid cavity of egg

b)Cell structure inactivated vaccine

c)Recombinant influenza vaccine

- Indication – all above age of 6 months
- C/I – immunocompromised states

2) Live spray vaccine

- Local & systemic immunity
- Efficacy – less

Treatment 00:09:23

- Amantadine
- Oseltamivir

Role of sialic acid receptor 00:09:45

2-6 sialic acid receptors	2-3 sialic acid receptors
• Seen in humans	• Avian
• Seen in URT	• Seen in intestinal epithelium
• Respiratory epithelium	• LRTI

Role of pigs

- Pigs → both receptors

↓

Infected by humans & avian strains

↓

Genetic reassortment

Avian Flu 00:11:33

- Birds – reservoir
- Pigs – mixing vessels
- H5N1
  - Transmission from birds to humans
  - The number of cases were less
  - Symptoms
    - URTI
    - Diarrhea
  - DOC – Oseltamivir

HINI (swine flu)

- 4 strains
- Transmission – human to human
- High morbidity
- Pneumonia – bacterial (staphylococcus), diarrhea
- Treatment – oseltamivir
- Genetic shift
  - Can cause pandemics
  - Seen once in 10 – 20 years → High morbidity
  - Risk group – old age, immunocompromised state and respiratory compromise

Von Magnus phenomenon 00:16:07

- Replication is not seen by but HA titer is high

POLIO VIRUS

Picorna viruses 00:00:10

- 2 groups
  - Enteroviruses - Poliovirus
  - Rhinoviruses - Common cold

Poliovirus 00:00:35

- Serotypes
  - Type - 1 – endemic & also capable of epidemic potential
  - Type - 2 – Can be eliminated
  - Type - 3
- Transmission – few - oral route
- Site of action - Anterior horn cell of spinal cord
- Receptor for binding CD 155 receptor

Clinical manifestations

- Inapparent infection
- Abortive infection
- Non-paralytic
- Paralytic infection
  - Flaccid paralysis
  - It affects proximally
  - Hip → extremities

↓

Tripod sign

Lab diagnosis: 00:02:45

- Route of spread – Hematogenous

Lab diagnosis

Specimens – Blood, throat swab, CSF, feces

Virus isolation	Antibody detection	Molecular methods
Primary monkey Kidney cell line ↓ Crenation or degeneration of entire cell line	• CFT	↓ RT-PCR • Sensitive & specific • Differentiates between wild strains ↓ VAPP VDPV

Polio vaccines: 00:04:37

	Salk vaccine (killed)	**Sabin (oral)**
Preparation	Killed vaccine formalin inactivated (MKG)	Oral • 3 serotypes • Bivalent 1,3 • Monovalent
Schedule	6 weeks	0, 6, 10, 14 weeks Booster – 16 -24 wks
Economy	Expensive	Relatively cheap
C/I	No C/I	Immunocompromised
Efficacy is affected by	–	• Enteroviruses • Diarrheal disease

		• Breast feeding
Duration of protection	Short lived	Long lasting
Herd immunity	–	++
local immunity	–	IgA
VAPP, VDPV	–	+
Storage conditions	Stringent storage condition	• -20° • Transported in cold chain

VAPP

- Serotype – sabin 3 – sabin 2
- Seen because of administration of OPV – 1st dose
- Mutation occurs
- OPV – like isolated - <1% OPV

VDPV – serotype – type 2 – type 1

1. Circulating VDPV's
    - Causes outbreaks
    - Prevention by OPV coverage
2. Immunodeficiency associated VDPV's
    - Shed the virus without symptoms
    - Affected by OPV coverage
3. Ambiguous VDPV's
    - Heterogenous

Pulse polio program: 00:10:50

- Given below the age of 5 years
- During winter season
- All, irrespective of immunization schedule 2 doses are given 6 weeks apart
- Provides herd immunity
- 2 countries where polio is endemic

  a. Pakistan

  b. Afghanistan

COXSACKIE VIRUS, ENTEROVIRUS, ECHINOVIRUS, RHINOVIRUS

Coxsackie virus 00:00:10

Coxsackie virus

Coxsackie A	Intracerebral inoculation in sucking mice	Coxsackie B
• Flaccid paralysis • Generalized myositis • Clinical - A – Aseptic meningitis - H – Herpengina - H – Hand – foot – mouth disease - H – hemorrhagic conjunctivitis		• Spastic paralysis • Focal myositis necrosis of brown fat • Clinical - Aseptic meningitis - Pericarditis - Myocarditis - Bornholm disease / epidemic - Pancreatitis

Rhinovirus 00:03:03

- Common cold
- Acid labile
- Usually seen in URT → optimum temperature is 33°C
- Receptor for bindings – I – Cam 1
- IP – 2 – 4 days

↓

- Nasal congestion

  - Nasal discharge
  - Sneezing
- Rhinovirus – more than 100 sero types

  ↓

  Type – specific immunity

Lab diagnosis

- Virus isolation – monkey kidney cell line used
- Antigen detection – ELISA
- Molecular method – PCR

Enterovirus 00:06:10

Enterovirus

70

↓

Responsible for acute hemorrhagic conjunctivitis

↓

Self-limiting condition

71

↓

Hand – foot – mouth disease

72

↓

HAV (hepatitis A)

Echovirus 00:07:00

- Responsible for causing aseptic meningitis
- Encephalitis
- Fever with rash
- Ocular – conjunctivitis

INTRODUCTION TO MEDIAL MYCOLOGY

- Medical mycology – causes mycoses
- Fungi
  - Eukaryotic
  - Cell wall – Chitin, β D glucan
  - Cell membrane – Ergosterol
- Antifungals
  - Mode of action

β Glucan	Ergosterol	DNA synthesis
↓ Echinocandins (They inhibit β glucan synthesis)	↓ A – Alylamines A – P – Polygenes (A, P – inhibit ergosterol)	↓ Consists of antimetabolites ↓ Flucytosine (inhibits DNA synthesis)

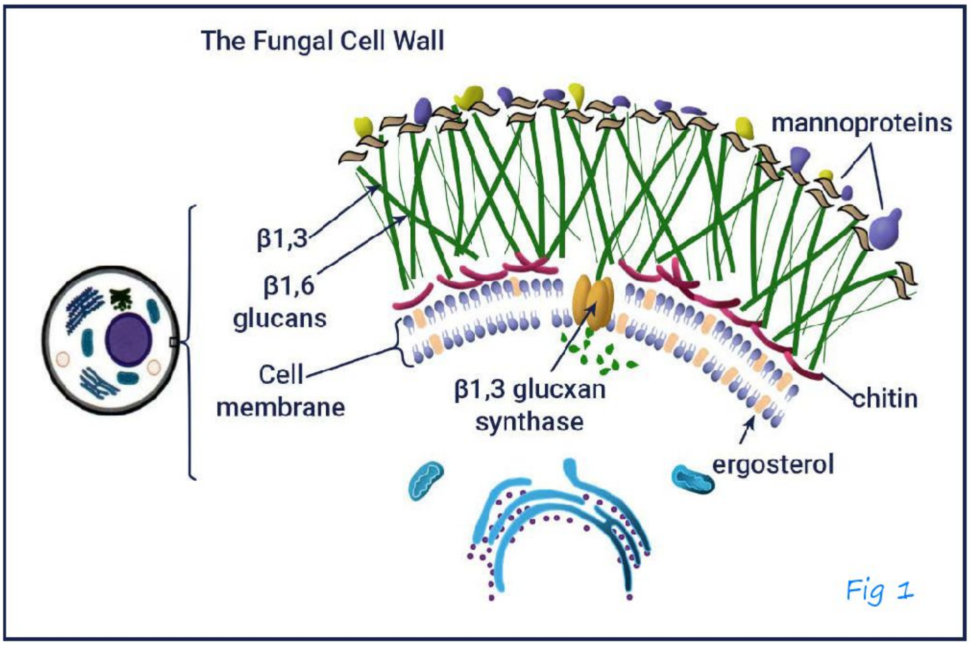

The Fungal Cell Wall
mannoproteins
β1,3
β1,6
glucans
Cell membrane
β1,3 glucxan synthase
chitin
ergosterol

Fig 1

- β- Glucan test is non- invasive test that is developed for invasive conditions like invasive like invasive aspergillosis
- Cryptococcus & Zygomycetes → they do not produce β Glucan

Classification

I) Morphological

II)Taxonomical

III) Clinical condition

I) Morphological classification

- Yeast (True yeast)

  e.g., Cryptococcus, Sacromycetes
- Yeast like

  Constriction is a characteristic feature
- Molds – branching filament are seen

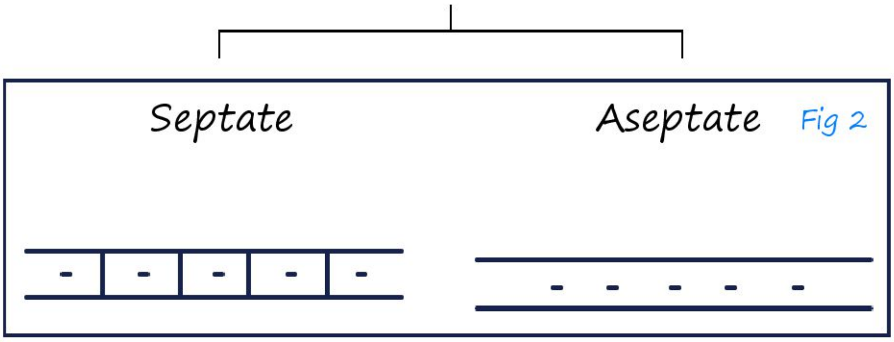

Septate
Aseptate

Fig 2

- Mycelium – mass of filaments

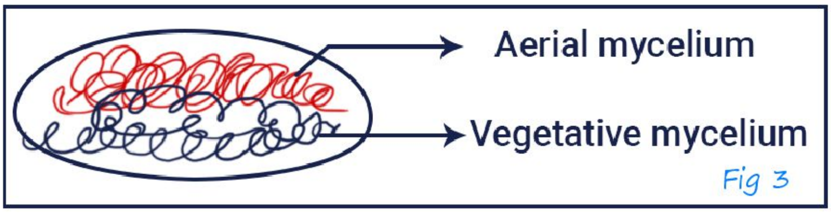

Aerial mycelium
Vegetative mycelium

Fig 3

- Dimorphic fungi
  - At 26°C hyphal forms
  - At 37°C yeast forms

II)Taxonomical classification: sexual spores

Zygomycota	Ascomycota	Basidiomycota	Deuteromycota
↓ Zygospores ↓ Aseptate hyphae E.g., mucor Rhizopus	↓ Ascospores ↓ septate hyphae Aspergillus	↓ Eg: Cryptococcus	↓ Fungi imperfecti • medically important fungi • sexual state is not defined

III) Clinical classification

Superficial mycosis	Subcutaneous mycosis	Systemic mycosis	Opportunistic mycosis
It involves • Skin • Nail • Hair • Mucosa	• It involves skin with subcutaneous tissue • Direct inoculation	Multiple organ involvement	Commences causes infections

Lab diagnosis

Routine microbiology	Histopathology	
• KOH mount – Skin – 10% – Nail, – Tissue — 20-40% • India INK preparation, Nigrosin — Negative staining (here, the background is stained) • Calcofluor white	PAS stain ↓ Only stain live fungi	GMS stain ↓ Stains both live & dead fungi

Culture

- Grown on SDA (P^H on SDA – 5.6%)
- (P^H can vary to 7.2% – Emmon's modification)

Others

- BHI Agar
- Corn meal Agar
- Bird seed Agar
- Chrom Agar

Incubation

- BOD – biological oxygen demand
- For Dimorphic fungi
  - 25°C
  - 37°C

- It takes 4-6 weeks of grow
- LPCB mount → (used to visualize)

Lactic acid

Disinfectant phenol

Cotton blue

Can be used to demonstrate hyphae as well fruiting bodies

Slide culture

- Incubated at 25°C – 27°C
- Dalmau's plate – for demonstration chlamydospores in candida

Antifungals

β - D- Glucan	Ergosterol	DNA synthesis
↓ Inhibition of synthesis ↓ Echinocandins	Ergosterol ↓ Inhibition of synthesis ↓ A- Azoles A-Allylamines P- Polyenes E- AAP	↓ Antimetabolites ↓ Flucytosine

MYCOPLASMA

- It is the smallest microble
- Capable of independent survival - free living state in the environment
- Can be grown on artificial medium
- It resembles viruses
  - Filtered by bacterial filters
- Other factors

i) It is deficient of cell wall

↓

3 layered cell membrane which contains steroid

ii) Cannot use β - lactam antibiotics because of deficient cell wall

iii) Most common contaminants in cell culture

iv) They are pleomorphic

v) Poorly gram negative

Mycoplasma species

1) M. pneumonia
2) M. Genitalium
3) M. Hominis
4) Urea plasma, urealyticum
5) U. Parvum

Difference between mycoplasma & L- forms

00:04:19

Mycoplasma	L-form
• Cell wall deficient ↓ Permanent • Cell membrane has sterol • Filtrable 'cell wall precursor - Peptidoglycan • Can reduce infection	• Cell deficient forms • Spontaneously or due to use of β- lactam antibiotics • Sterol is absent • Non-filtrable • No peptidoglycan • Can lead to persistence of infection

Mycoplasma pneumonia

00:07:00

- Atypical pneumonia
- Mode of infection - respiratory droplets
- Incubation period – 2 - 4 weeks

Clinical spectrum

URTI
- Tracheobronchitis
- Otitis media
- Pharyngitis

Pulmonary
- Eaton agent pneumonia or walking pneumonia

Extra-pulmonary
- Carditis
  - Pericarditis
  - Myocarditis
- Reactive arthritis
- Anaemia
- CNS
  - GBS
  - Aseptic meningitis

Lab diagnosis 00:09:46

Culture: PPLO medium

PPLO agar	PPLO broth
• Penicillin • Horse serum ↓ Fried egg colonies ↓ Central granular growth surrounded by peripheral Translucent growth	• Broth • Penicillin • Phenol red indicator ↓ Red turns yellow due to break down of RCBs (fermentation of glucose

Handlens

Diene's method
↓
Agar plate is flooded with blue with azure
↓
Royal blue colour is seen

Other media

SP-4 medium

Hay lick's modified medium

Other methods

- Hemolytic colonies
- Hemadsorption ↓ Guinea pig RBC's
- Tetrazolium test ↓ formazan
- Growth is inhibited by specific antisera

Molecular methods

- PCR ↓ Detect for particular antigen
- Multiplex PCR ↓ Atypical pneumonia

Antigens 00:15:38

Antigens

- Glycolipid antigen ↓ It cross reacts Heterophile antigens
    - Cold agglutination test ↓
        - It is performed at 4°C
        - 'I' antigen-blood group 'O'
    - Streptococcus MG test ↓ Cross reacts with carbohydrate group F streptococcus
- P1 Adhesin antigen ↓ Used for detection of antigen & antibodies
    - ELISA
    - CFT

Treatment 00:18:06

- Azithromycin
- Doxycycline
- Levofloxacin

Urogenital infections 00:18:40

- They colonize the female genital tract
- Infections-sexual route
  - Transmission during birth

Clinical spectrum

- PID
- Non - gonococcal urethritis
- Epididymitis
- Wound infections
- Brain abscess

Treatment

1) Azithromycin
2) Doxycycline

KLEBSIELLEAE

i) Klebsiella

ii)Serratia

iii) Enterobacter

iv) Hafnia

v)Pantoea

KLEBISELLA 00:01:07

- K. pneumoniae
- K. oxytoca
- Most common - lobar pneumonia

  ↓

  Red currant jelly sputum

- It is a capsulated organism
- It is non - motile
- Colonies are dome shaped
- Infections caused
- Nosocomial infection
  - Respiratory infection
  - UTI
  - Wound infection
  - Septicaemia
  - Endocarditis
  - Burns
- K. pneumonia ozaena → atrophic Rhinitis

  ↓

Foal smelling discharge

- K. pneumonia Rhinoscleromatis → Rhinoscleroma

↓

Deformity related to nose

Lab diagnosis 00:04:25

- Gram staining - plump gram - negative bacilli
- Cuture-MAC - lactose fermenter

↓

"dome shaped" colonies

- Biochemicals

		K. pneumonia	K.oxytoca
Bio chemicals	a) Indole b) MR c) VP d) Sugar e) TSI f) Urease Citrate	Negative Negative Positive Ferment sugar except sucrose A/A with abundant Gas with H2S Positive Positive	Positive

	Indole	MR	Vi	Citrate
E. coli	+	+	–	–
Klebsiella	–	–	+	+

PROTEUS

- Belongs to the tribe – proteac
- 3 genera
  - Proteus
  - Morgan Ella
  - Providentia
- Proteus- PPA positive
- Phenylalanine → Phenyl pyruvic acid

↓

Enzyme

(Phenylalanine deaminase)

- It is pleomorphic - coccobacilli

Bacillary form

Named after Greek God proteus

- It shows a phenomenon called as swarming
- It is urease positive

- Odour-fishy/seminal odour
- Species responsible for disease
  - P. Mirabilis
  - P. Vulgaris

Human disease

Proteus pathogenesis 00:03:30

Saprophytes

↓

Survive on decaying matter

Nosocomial infections

Hospital setting

Others

- Struvite stones
- Proteus produce → urease enzyme
- Urea → ammonia

↓

Damages epithelium

↓

Phosphate

↓

Stones

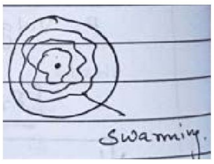
Antigens 00:05:30

1. O-Antigen - somatic antigen → present in non-flagellated proteus species
2. H-Antigen- present in flagellated species

- Weil-felix test: somatic antigens from non-motile strains (X-strains) → cross reacts with Rickettial antigen
- Heterophile antibodies in patients' serum
  - X19 ⎤
  - XO ⎦ P. Vulgaris
  - XK -P. mirabilis

Lab diagnosis 00:07:50

- Gram staining - pleomorphic gram-negative organisms
- Odour- seminal odour
- Blood agar - swarming
  - Uniform
  - Concentric



↓

From margin of colony

↓

Sparsely flagellated vegetative cells

↓

Converted to highly flagellated swarm cells

↓

Swarm cells tend to move to uninoculated areas of the medium

- MAC or CLED → swarming is inhibited

- Inhibit swarming
  - Increase % of agar to 6%
  - Others-Boric acid and CLED, MAC→inhibit swarming
- Biochemical reactions
  - Urease-positive
  - Citrate -variable
  - MR-positive

Indole	P. Mirabilis	P. Vulgaris
	Negative	Positive-red ring

- Proteus tends to be commensal→present in moist areas & intestine

Morgaella morganii 00:14:23

- Isolated from human faces
- Indole positive
- Citrate- Variable
- Urease-positive
- TSI-K/A with gas but no H2S
- It does not show swarming

Providentia 00:16:00

- 5 species →no swarming
- Causes nosocomial infections

  - UTI
  - Burns
  - Wound infections
- MAC- NLF colonies
- Antibiotic resistance

↓

Intrinsic resistance→nitrofurantoin polymyxin

- It is also resistant to β- lactum antibiotics because of AMP β-lactamases

Other organism showing swarming 00:19:04

a)Cl. Tetani

b)Vibrio parahaemolyticus

c)Serratia

Diene's phenomenon 00:19:45

i) 2 strains are inoculated

↓

Merging of two strains without line of demarcation

↓

2 strains are identical

ii)2 non-identical strain

- Line of demarcation is seen

SERRATIA

- See mostly as saprophytes
- Can cause nosocomial infections
  - Respiratory infections
  - It produces a pigment which is red in colour

    ↓

    Pseudohemoptysis
  - UTI
  - Wound infections
  - Burns
- Produces pigment-prodigiosin

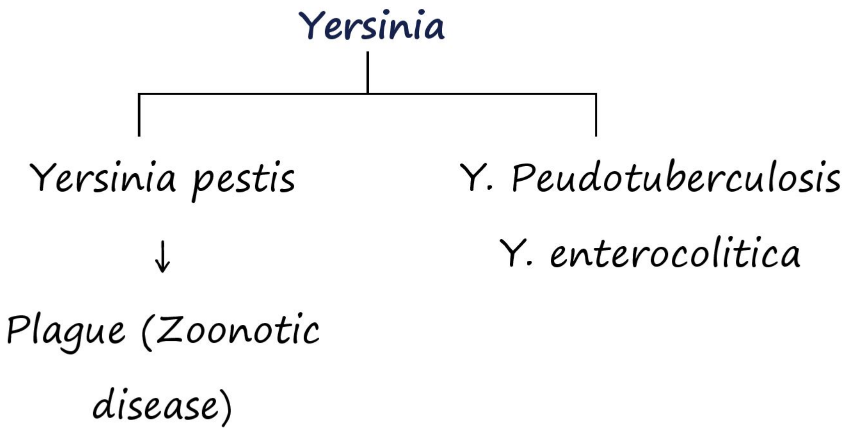
AliaTafreeh.com

Diffusible pigment	Non-diffusible pigment
• Pseudomonas aeruginosa ↓ Because of diffusible pigments - Pyocyanin - Pyorubin - Pyoverdine	• Pigment is confined only to the colony • Staphylococcus aureus ↓ Produces a golden yellow pigment ↓ "Carotene" • Serratia

- Serratia-resistant to Colistin (drug)

YERSINA PESTIS AND YERSINOSIS

Yersinia 00:00:15



Yersinia

Yersinia pestis
↓
Plague (Zoonotic disease)

Y. Peudotuberculosis
Y. enterocolitica

Epidemiology 00:01:00

- Reservoir – Rodents, Gerbils, Field mice, Ban dicoote
- Vector – Rat flea (Xenopsylla cheopis, Xenopsylla astia, Xenopsyla brasliemsis)
- Human (Pulex irritans)
- Cheop's index – Average of X. cheopis per rate >1 → Plague prone area

Virulence factors 00:03:05

- F$_1$ antigen – Inhibits phagocytosis
- Phospholipases D
- PH^6 antigen
- Siderophore
- Lipopolysaccharide
- Pigmentation

Clinical spectrum 00:04:05

1.Bubonic plague

Transmission – Bite of rate flex

↓

Local lymphatics

↓

Lymph nodes

↓

- Fever
- Painful lymphodenitis
- Bubous – Painful lymphodenitis
- Adults – Inguinal lymphnodes
- Children – Cervical lymph nodes
- Incubation period – 2-7days

2.Pneumonic plague

- Transmission – Inhalation of droplets containing coccobacillis
- More fatal
- Symptoms – Cough & fever
- Biterrorism
- Incubation period – 1-3days

3.Septicemic plague

- Causes hemorrhages on body

↓

Gangrene

↓

"Black death"

Lab diagnosis 00:07:46

1.Gram staining

- Gram negative coccobacillic with in capsule
- Wayson staining
  - Bipolar staining
  - Safety pin appearance

2.Culture

- Blood agar → Brown coloured colonies due to absorption of hemin pigment
- chocolate agar
- Nutrient broth
  - Granular turbidity
  - Nutrient broth with ghee → Stalachite growth

3.CIN agar

Gram staining

- Pleomorophic bacteria

Motility

Y. Pesitis

- Non-mofile at 25° c & 37° c

Y. Pseudotuberculosis

Y. enterocolitica

- Motile at 25° C
- Non-motile at 37° C

Treatment 00:11:37

- Streptomycin
- Gentamycin
- Doxycycline

Prevention 00:11:51

- Prevention of rodents
- Control of rat flea
- Isolation of and treatment of patients & contacts
- Chemoprophylaxis
  - Doxycycline
  - Tetracycline

  7days

Vaccination 00:12:41

- Formalin killed vaccine
  - Prepared in haffkine institute – Mumbai
  - C/I – Less than 6months of age
- Live attenuated vaccine
- Subunit vaccine

Yersiniosis 00:13:47

- It is associated with in GI illness
- Y. Pseudotuberculosis & Y. Enterocolitica are responsible for yersiniosis

Clinical spectrum 00:14:32

- Gastroenteritis
- Superantigen mitogen → Scarlet – Like fever & Izumi fever
- Pseudo appendicitis → Mimics appendicitis
- Post infection
  - Reactive arthritis
  - Erythema nodasum
  - Grave's disease → Y. Enterocolitica

Lab diagnosis 00:16:15

Blood	**Lymph node aspirate**	**Soils feces**	**Biochemical test and others**
• BHI broth	• Blood agar translucent colonies with in copper beaten • MAC-NLF colonies	• CIN agar	• Cold enrichment • Differential motility – Motile at 22-25° C – Motile at 37° C

Treatment 00:18:35

- Fluoroquinolones
- Cephalosporins

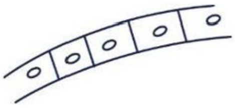
BACILLUS

Bacillus: 00:00:10

- Gram positive, spore bearing organisms

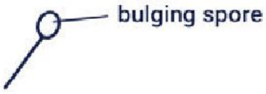
1.Bacillus

  - Obligate aerobe
  - Forms non -bulging spores



2.Clostridium

  - Obligate anaerobes
  - Bulging spores



- Bacillus is gram positive, rectangular rods, sporing with non-bulging spores
- Bacillus
  - B. Anthracis
  - B. Cereus
- Most of the bacillus are motile and non-capsulated
- Motility-peritrichous motility. Except B. anthracis
- B. anthracis is non-motile and capsulated
- B. anthracis causes zoonotic disease – Anthrax
- B. anthracis – Bioterrorism

Virulence factor: 00:04:45

A. Capsule

- Polypeptide capsule made up of polyglutamate

B.Toxin

- 3 fragments

Edema factor	**Protective factor**	**Lethal factor**
- Active fragment - Increase host with cAMP - Edema	- It is called a binding factor (fragment) - Allows another fragment to enter	- Cell death

Clinical manifestation: 00:07:20

1.Animal anthrax

Spores present in the soil is ingested by herbivores like cattle

↓

They shed bacilli from nose, rectum, mouth

↓

Bacilli sporulate in soil

↓

2.Human anthrax

- Contact with soil due to abraded skin (most common route) can cause cutaneous anthrax
- Inhalation of spores – causes pulmonary anthrax
- Consumption of animal carcase- can lead to GI symptoms – blood diarrhea

Clinical conditions: 00:10:32

Cutaneous Anthrax	Pulmonary Anthrax
• Abraded skin in contact to spores	• Route – inhalational
• Causes malignant pustules papule → painless pustule ↓ Necrotic Eschar covering it	• Condition – hemorrhagic pneumonia ↓ Spread through lymphatics ↓ Cause lymphadenitis, meningitis
• It is also called as hide porter's disease	• Also called as wool sorter's disease

Lab diagnosis: 00:13:42

Specimen – CSF, sputum, pus swab

A. Gram staining

- Gram positive rectangular Rodes with non-bulging spores
- Capsule demonstration
  - Most common Fadyean reaction
  - Staining methylene blue for 30 sec

↓

Purplish material surrounding the bacilli

- Demonstration of spores

Ashby's method   0.25% H_2SO_4

- Use malachite green

- Demonstrate lipid granules

↓

Sudan B black (Burton's methos)

B.Ascoli Thermoprecipitation test

Tissue is ground finally

↓

Antigen is overlayed with specific antisera

↓

Ring of precipitation where the two liquids meet

C.Culture

1.Nutrient agar

- It has greish frosted colonies

↓

When you focus the edge of the colony under low microscope

↓

Matted hair appearance because of long chain of bacilli

↓

This appearance is known as medusa head appearance

2.Blood agar

- Dry, wrinkled, non-hemolytic colonies

3.Gelatin agar

- Inverted fir tree appearance

4.Selective medium

a. PLET medium

b. Selective medium with penicillin

↓

String of pearl appearance

Treatment: 00:22:12

- Ciprofloxacin
- Clindamycin
- Doxycycline

Vaccine: 00:22:35

1.Stem vaccine

- Live attenuated vaccine
- Used in animals
- Efficiency – 1 year
- Efficacy – 1 year
- Not used in humans

2.Alum precipitated, toxoid vaccine

- Used in humans
- Occupational risk
- 3 doses
  - 1
  - 6 weeks
  - 6 months

Bacillus cereus: 00:24:55

- This is isolated from soil, vegetable → beans, cattle, poultry etc.
- The clinical conditions are

1.Food poisoning

Emetic	Diarrhea
• Preformed toxin	• Toxin is produced in intestine
• Heat labile	• Heat stable
• Resembles staphylococcus enterotoxin	• Resembles Cl. Perfringens enterotoxin
• Incubation period – 6-8 hours	• Incubation period – 8-16 hours

2.Ophthalmological

- Keratitis
- Panophthalmitis

3.Others

- Bacteremia
- Meningitis
- Septicemia

Lab diagnosis: 00:28:22

- Sample – feaces
  - MYPA agar
  - PEMBA agar

Treatment: 00:29:00

- Ciprofloxacin
- Clindamycin

Others: 00:29:13

- Bacillus Thuringiensis
  - It is a larvicidal agent

    B. Stereothermophilis
    B. Subtilis
    B. Globigi

    Biological indications for sterilizing

CORYNEBACTERIUM DIPHTHERIA

- Gram-positive bacilli – non capsulated & non-sporing

Virulence factor 00:01:50

Diphtheria toxin

A fragment
↓
Active fragment
↓
ADP Ribosylation of EF-2
↓
Inhibition of EF -2
↓
It inhibits translation of protein synthesis

B fragment
↓
Binding Fragment for Binding to Host Epithelium

Pathogenicity 00:02:38

- The organism acts locally
  ↓
  But toxin-mediated damage is there

Clinical spectrum 00:03:18

A). M.C – Tonsils & pharynx	**B) cutaneous**
Toxin causes inflammation ↓ Necrosis ↓ Ulcer are formed ↓ Ulcers are covered by pseudo membrane which bleeds on removal ↓ This pseudo membrane spreads to larynx & bronchial airway ↓ Pt. complaints of stridor, difficulty in breathing, foul smell ↓ Emergency tracheostomy	• Punched out lesions covered with pseudo membrane • This is caused by the organism and not toxin **C) Systemic manifestations** • Myocarditis • Polyneuropathy

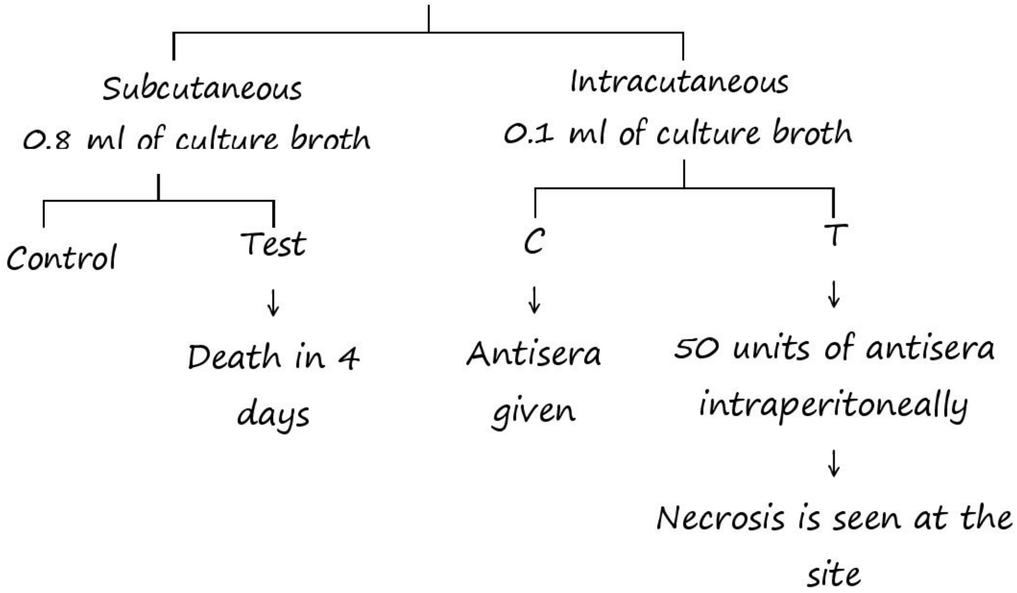
Lab diagnosis

Gram staining	Culture	Selective medium	
• Produces gram positive bacilli, Not evenly stained • Arranged in cuneiform pattern or Chinese letter pattern • Albert sating – Green bacilli with bluish – black volutin granules	Loeffler's serum slope – Nutrient agar + Horse/sheep serum + Glucose Growth seen in 4-6 hrs White glistening colonises seen • Advantage – Growth is detected quickly • Disadvantage – Commensal organism also grows	Hoyle's PTA • Tellurite is converted to tellurium • Black coloured colonies • Adv: commensals do not grow	Modified PTA with cysteine • Black to brown colonies with halo • Cysteine is broken down by cysteinase enzyme

Biochemical reactions:

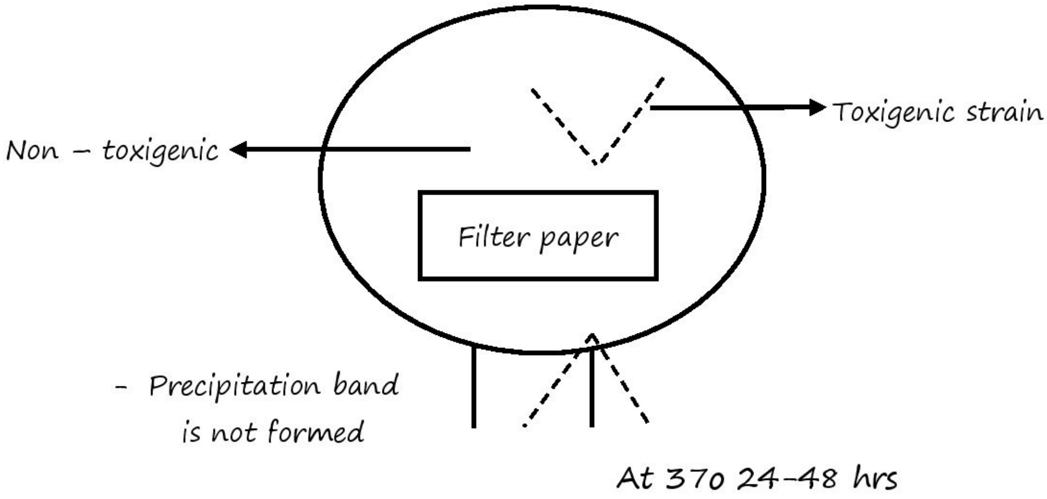
- Hiss serum sugar
- Pyrazinamide test +ve
- Urease test -ve

Animal inoculation 00:11:40


Subcutaneous
0.8 ml of culture broth
Control
Test
Death in 4 days
Intracutaneous
0.1 ml of culture broth
C
T
Antisera given
50 units of antisera intraperitoneally
Necrosis is seen at the site

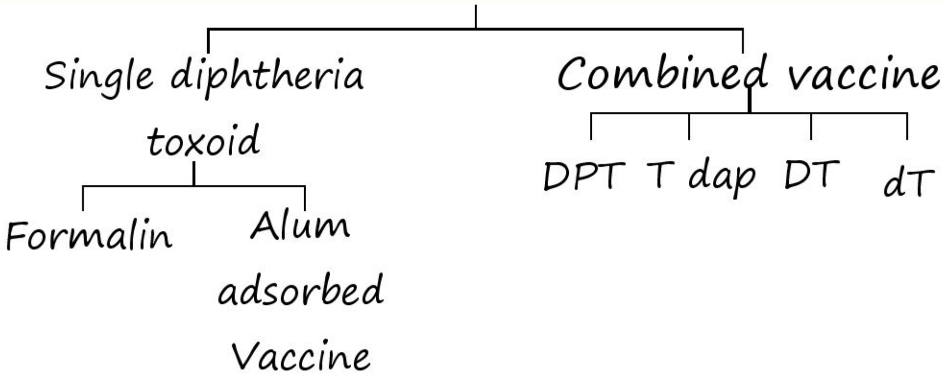
Ellk's Gel precipitation test 00:13:30

- Take filter paper soaked in antitoxin & is placed in a culture media of 20% horse serum
- The streak suspected strain at right angles


Toxigenic strain
Non – toxigenic
Filter paper
- Precipitation band is not formed
At 37o 24-48 hrs

- Identification of tox gene by PCR
- Diphtheria toxin by ELISA

Vaccination 00:15:32


Single diphtheria toxoid
Formalin
Alum adsorbed Vaccine
Combined vaccine
DPT
T dap
DT
dT

- DPT – Diphtheria

  Pertussis ← acts as adjuvant Tetanus
- 5 doses – 6, 10, 14, months
- Booster
  - 16 - 24 months
  - 5 yrs
- Preservative -thiomersal
- Site – anterolateral aspect of thigh
- DPT Vaccine should never be freezed
- Dt – given after age of 12years
- DPT Should not be given after 6 years of age because it can cause encephalitis

  ↓

  Instead give TDap -acellular pertussis

Schick test 00:19:25

- Obsolete now
- Inject toxin & intra – dermally

↓

Pt develops erythema & induration

↓

Considered to be susceptible

↓

Then vaccination is given

LISTERIA MONOCYTOGENES

Listeria monocytogenes

- It is a saprophyte
- Food borne pathogen
- Extremes of age & mainly affects pregnant women
- Mode of spread – contaminated food

↓

Refrigerated food – cheese, salads, or other dairy products

↓

Listeria survives refrigeration at 4°c

- Listeria is a facultative interacellular organism

↓

It enters intestinal epithelium by internaline

↓

Phagosome → listerolysin O

Clinical spectrum 00:03:00

1.In Neonates

- They acquire infection through maternal genital tract or from the environment

  a. Early onset – less than 5 days

  - Maternal genital tract – mode of infection
  - Septicaemia

- Granulomatous infantiseptica
- Granulomas on skin, spleen live
- > 30% mortality

b. Late onset is more than 5 days of birth

- Acquired from environment
- Meningitis
- <10% mortality

2. Pregnant woman
   - Can cause abortion pre-term labour
3. In Adults
   - It can cause
     - Bacteraemia
     - gastroeteritis
     - Septicaemia
     - Meningitis

↓

Immunocompromised patients

Lab diagnosis 00:06:22

Gram staining

- Gram positive coccobacilli

Motility

- Differential motility
  - 25^0 Motile
    - Tumbling motility
    - Vibrio-darting motility
    - Cl tetani – stately motility
    - Giardia – falling leaf motility
  - 37^0 Nonmotile

Culture

- Blood ager
  - βHemdytic colonies
- Chocolate Auger
- Selective media – PALCAM M

Biochemicals

- Catalase +ve
- Listeria monocytogenes – CAMP test +ve
- Can grow in 10% Naci or even when PH is increased
- Increase the isolation when it is refrigerated

GRAM POSITIVE FILAMENTOUS BACTERIA, NOCARDIA & ACTINOMYCES

Actinomcetes 00:0020

- Gram positive non-sporing, no—capsulated bacteria arranged in chains or seen as branching filaments

Genera

Actinomyces	**Nocardia**	**Actinomadura**	**Streptomyces**	**Thermophilic actinomyces**
• Anaerobe & non-acid-fast	• Aerobe & acid-fast	• Aerobe & non-acid-fast	• Aerobe & non-acid-fast	

Clinical manifestation 00:03:17

Actinomyces

- This is an endogenous infection
- Present in the oral cavity
- It causes granulomatous conditions
  - Multiple discharging
  - Sinuses
  - Tumor – like appearance
  - Granules → micro-colonies of bacteria

Spectrum

Cervi facial actinomycosis	**Intestinal**	**Genitourinary**	**Brain abscess**	**Soft tissue**
Most common H/O debtal manipulation ↓ Lumpy jaw	Spillover of commensal flora	Infection of IUD		Infection with involvement if bone

Lab diagnosis 00:06:48

- Specimen
  - Pus
  - BAL fluid
  - Sputum
- When you want to identify the species, it can be done by
  - Biochemicals
  - GLC
  - PCR

Nocardia 00:12:00

- Gram positive, filamentous bacilli
- Source – exogenous
- Nocardia → environment
  - Vegetation
  - Debris
  - Soli

- Most important risk factor → decrees cell mediated immunity
    - Increase incidence in patients with HIV on corticosteroid history

Pathogenesis 00:13:45

Pathogenesis

- Inhalation
    - Inhalation of fragmented bacilli
- Transcutaneous inoculation
    - Traumatic inoculation

      ↓
    - Actinomycetoma

Most important species

- N Brasiliense's
- N asteroids

Clinical spectrum 00:15:08

Pulmonary nocardiosis

- Inhalation
- Lobar pneumonia → Fever with thick sputum

Extra – pulmonary

- Disseminator through blood

  ↓

  Subacute brain abscess (superterrestrial)
- MC – Brain
  - Kidney
  - Bones

Mycetoma

- Tumor – like
- Multiple discharging sinuses
- Granules

Lab diagnosis 00:17:23

Specimen – sputum, BAL, PUS

GRAM STAINIG (Brown-Bren modification)

↓

G.P Filamentous bacilli

Modified ZN staining 1% $H_2 So_4$

↓

Aerobic

Partially acid-fast

↓

Red coloured filamentous bacilli

Culture

↓

37°c 2-4 weeks

- Prefinalist technique

  ↓

  Ability of Nocardia to use paraffin as sole source of carbon
- LJ Medium – globous growth
- Buffered yeast extract
- SDA
- BHI

SDA, BHI – Growth is wrinkled orange pink colonies (pigmented)

Treatment 00:21:47

- Sulphonamide
- Cotrimoxazole

Actinometer (Red granules) 00:22:10

- A pellet Teri
- A actinometer

Lab diagnosis

- Gram staining
  - Filamentous gram – positive bacilli
- Culture
  - Molar tooth appearance

Treatment

- Amikacin
- Imipenem

STAPHYLOCOCCUS

Staphylococcus: 00:00:20

- Gram positive cocci

Micrococcaceae
- Staphylococcus
- Micrococcus

Streptococcaceae
- Streptococcus
- Enterococcus
- Pneumococcus

- How to differentiate microccaceae & streptococcaceae?

	M	S
1) Catalase test	Positive	Negative
2) Gram staining	GPC in clusters & tetrads	GPC in pairs & chains

Difference between staphylococcus & micrococcus: 00:04:07

	Staphylococcus	Micrococcus
A) O-oxidative	Fermentative	Oxidative

fermentative test		
B) F – Furazolidone	Sensitive	Resistant
C) B – Bacitracin	Resistant	Sensitive
D) Gram staining	GPC in clusters	GPC in tetrads

Staphylococcus aureus: 00:07:05

- GPC in clusters
- Catalase positive
- Coagulase positive
- Non – motile
- Facultative anaerobe

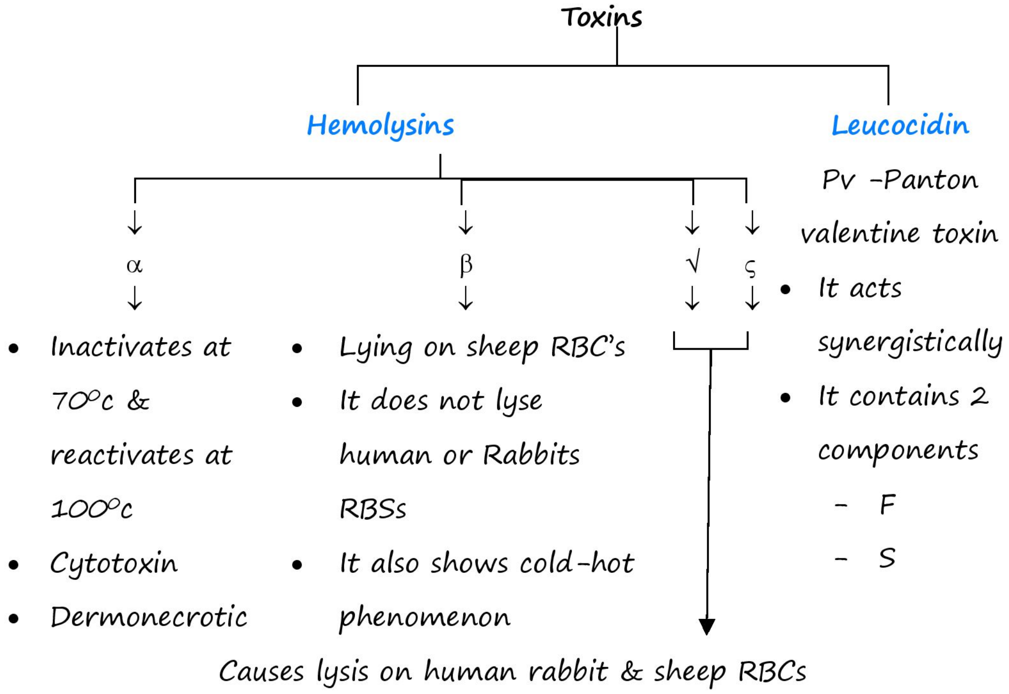
Virulence factors: 00:08:07

1.Cell wall factors

- Peptidoglycan
  - Provides cell wall integrity
  - Initiates inflammatory response
- Teichoic acid
  - Prevents opsonisation
  - Aide in attachment mucosal surface
- Clumping factors bound coagulase
  - Slide coagulase test positive

- Protein A negative
  - Responsible for conglutination
  - Binds for Fc region of IgG and leaves gab region free

2.Toxins



Toxins

Hemolysins

α
- Inactivates at 70°c & reactivates at 100°c
- Cytotoxin
- Dermonecrotic

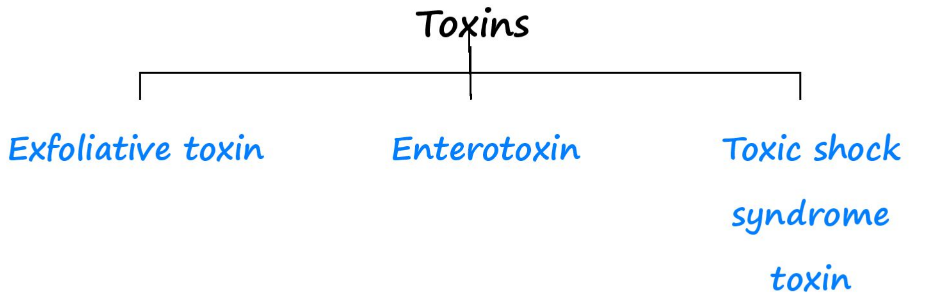
β
- Lying on sheep RBC's
- It does not lyse human or Rabbits RBSs
- It also shows cold-hot phenomenon

√ ς

Causes lysis on human rabbit & sheep RBCs

Leucocidin

Pv -Panton valentine toxin
- It acts synergistically
- It contains 2 components
  - F
  - S



Toxins

Exfoliative toxin
- Scaled skin syndrome
- Ritter's syndrome
- TEN – toxin epidermal necrolysis

Enterotoxin
- Food poisoning
  - Incubation period 1-6 hours
  - Common source is bakery product, Dairy product,

Toxic shock syndrome toxin
- Tampon users
- Symptoms
- Fever
- Hypotension

- Bullous impetigo (honey crusted lesions
- Pemphigus Neonatorum

processed meats & salads

- Most common symptom
  - Vomiting

↓

MOA – stimulation of vagus Nerve

+

Activates vomiting center in brain + increased intestinal peristalsis

3. Extracellular enzymes
   - Hyaluronidase
   - Phosphatase
   - DNAase
   - Lipase

Clinical spectrum: 00:21:25

a. Botryomycosis
   - Multiple discharging sinuses
   - Granular
   - Tumor – like growth

b. Osteomyelitis

c. Skin & soft tissue infections
   - Bullous impetigo
   - Furuncle

- Folliculitis
- Hidradenitis
- Suppurativa

d. Stitch abscess

e. Cellulitis

f. Pneumatocele in infants

g. Post viral pneumonia

h. Pharyngitis, Otitis, laryngitis

i. Food poisoning

Lab diagnosis: 0025:05

Gram staining	Culture		• Coagulants test • Catalase test ↔ +ve • Phosphatase test ↔ +ve • DNA ↔ +ve • Coagulase test – Tube +ve – Slide +ve
GPC in Clusters	Nutrient Agar • Golden yellow pigment (β - carotene)	Blood Agar • Hemolytic colonies	

Methycillin resistant staphylococcus Aureus: 00:28:21

- (MRSA)

  Mec A gene

  ↓

  Changes the penicillin binding site

  ↓

β lactam antibiotic becomes infective

- DOA – Vancomycin Linezolid
- In food handlers – carries
  - Take – nasal swabs – anterior nares
  - Groin
  - Axilla
- For Nasal carries – 2% Mupirocin ointment
- Skin carries – Chlorhexidine

Vancomycin Resistance: 00:31:35

- Vancomycin
  - Vancomycin intermediate S. Aureus (VISA) → Due to ↑ thickness of cell wall
  - Vancomycin Resistant S. Aureus (VRSA) → Van A gene.

Some imp points about S. Aureus: 00:32:50

- Pigment -β carotene – golden yellow pigment
- Selective media
  a. Mannitol sart agar
  b. Ludlam's media
- Resistance to antibiotics

β lactam antibiotics

Plasmid mediated (PM)

- β lactamases

  ↓

  Inactivates the β lactam ring

  ↓

  Overcomed by β - lactamases inhibitors
- PM changes occurs due to transduction

Chromosomal mediated

- MCC A gene

  ↓
- Penicillin binding protein (PBP)

  ↓

  PBP 2a

  ↓
- ↓ affinity of β lactam antibodies

- VISA is due to ↑sed thickness of cell wall
- VRSA is due to VAN A gene
- Pyomyositis in HIV – staphylococcus aurem
- Endocarditis

  1.Native valve Endocarditis
    - Hospital acquired → S. Aureus
    - Community acquired → S. Viridians

  2.Prosthetic valve endocarditis
    - Less than 12 months → S. Epidermis
    - More than 12 months → S. Viridians

  3.IV drug users
    - Most common cause

- S. Aureus

4.Subacute bacterial endocarditis – S. Viridians

- Community acquired MRSA
  - Necrotising pneumonia
  - Necrotising fasciitis
    ↓
    Most common - streptococcus
  - Water house Fredeichson syndrome
    ↓
    Most common - Meningococcus

STREPTOCOCCUS PART -1

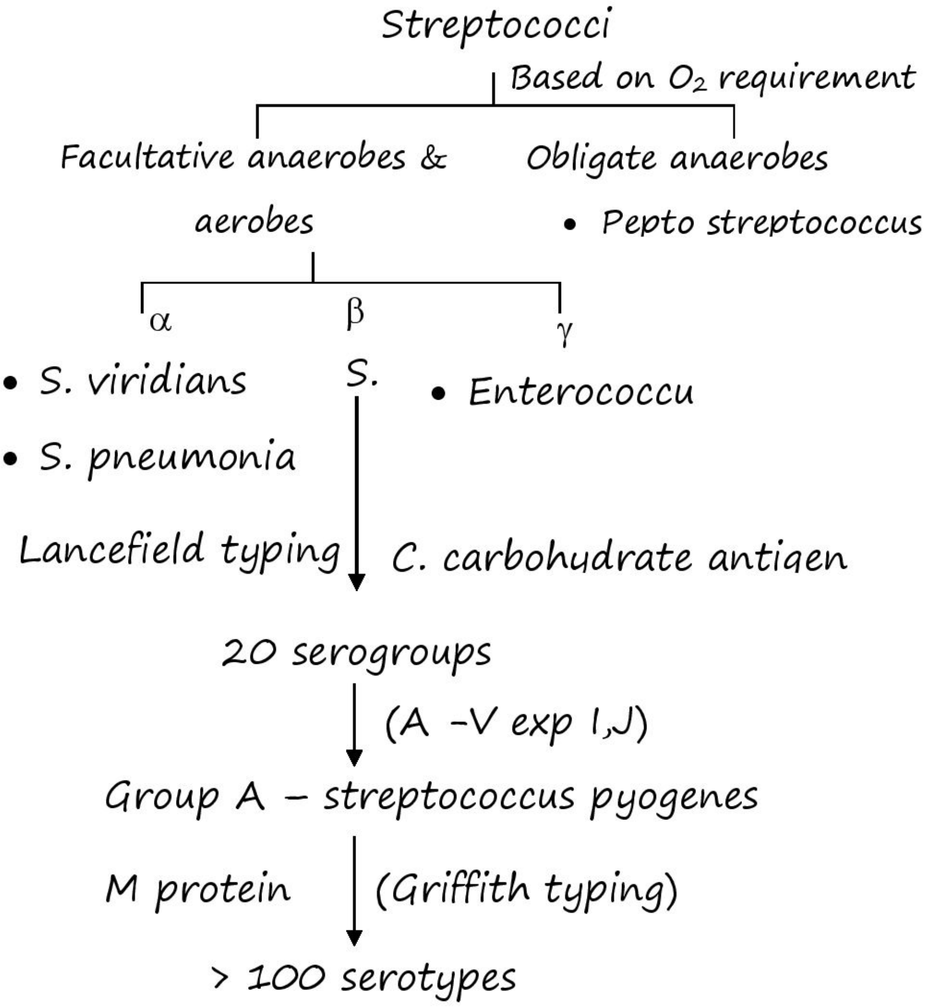
Streptococcus 00:00:10

- Gram positive in chains

Virulence factors 00:00:30

1) Cell wall factors
2) Toxin
3) Capsule
4) Enzymes

Differentiation of streptococci 00:01:20



Streptococci

Based on O_2 requirement

Facultative anaerobes & aerobes

Obligate anaerobes

- Pepto streptococcus

α

- S. viridians
- S. pneumonia

β

S.

Lancefield typing

C. carbohudrate antiqen

20 serogroups

(A -V exp I,J)

Group A – streptococcus pyogenes

M protein

(Griffith typing)

> 100 serotypes

γ

- Enterococcu

Virulence factors 00:04:45

I) Cell wall factors

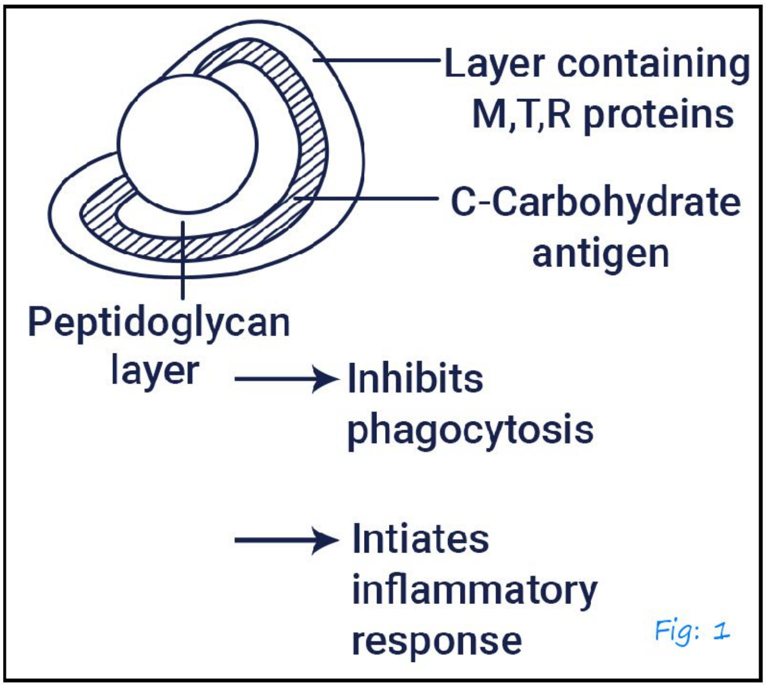

Layer containing M,T,R proteins
C-Carbohydrate antigen
Peptidoglycan layer
Inhibits phagocytosis
Intiates inflammatory response

Fig: 1

- M protein in outer layer is divided into 2 classes

↓

2 classes

I II

↑

- Antibodies to class I M protein is responsible for the pathogenesis of Rheumatic fever

II)Capsule

- Seen in mucoid strains
- Made up of Hyaluronic acid
- Not antigens

III) Toxins

1) Homolysis

Streptolycin - O
↓
- Oxygen labile
  Heat stable
- ASO antibodies are elevated in Rheumatic fever

Streptolycin - H
↓
- Oxygen stable
  Heat stable
- No diagnostic importance

2) SPE – streptococcal pyrogenic exotoxin

- Responsible for necrotising fasciitis, scarlet fever, toxic shock syndrome
- 3 antigenic subgroups
  - SPE – A
  - SPE – B
  - SPE – C

  Super antigens

IV) Enzymes

1) Streptokinase
↓
It converts plasminogen to plasmin
↓
Responsible for the breakdown of fibrin

- Indication – acute MI because it is a fibrinolytic

2) Hyaluronidase
↓
Helps in breakdown of hyaluronic acid

3) DNase

↓

Breakdown of DNA

Liquification of pus

- Empyema

Anti DNase

- Acute glomerulonephritis pyoderma

↓

Decrease ASO titre is low

4) NADase

STREPTOCOCCUS -PART 2

Clinical spectrum 00:00:10

Clinical spectrum

Suppurative

1) URTI – pharyngitis
2) Scarlet fever – SPE toxin
    - Sandpaper rash – rash is due to hypersensitivity to circulating antigens
    - Strawberry tongue
    - Pastia lines – Rash which involves skin folds
3) Soft tissues & skin infections
    - Impetigo

      ↓

      Honey comb crusted lesions

      ↓

      Painless lesions & not associated with fever
    - Cellulitis
    - Erysipelas

      ↓

      Orange peel texture of skin
4) Necrotising fasciitis (NF)
    - GAS – 60% NF

Non – suppurative

(Caused because of molecules mimicry)

↓

- Rheumatic fever
- PSGN
    - Circulating antibodies due to previous
    - Cross reactions with tissue antigens

↓

Non – suppurative causes

- Rheumatic fever
    - Molecular mimicry 'M – protein – cross reactivity
    - Diagnosed by modified Jones criteria
    - Diagnosis is also based on ASO tire →clavated
    - Common in women
- Post streptococcal glomerulonephritis (PSGN)
    - Age group – 5-12 years
    - Weeks after episodes of pyoderma or pharyngitis

- Superficial & deep facia involving muscles
  - Traumatic (most common cause)
  - Spill over → Involving abdominal wall

5) Bacteraemia

6) Sepsis→ 20 Necrotising fasciitis

7) Invasive conditions

- Deposition of antigen – antibody complexes in Glomerular basement membrane leading to complement activation

↓

- Also levels are low is diagnosed by anti-diagnose antibodies

- Others
  - Guttate psoriasis
  - Reactive arthritis
  - PANDAS (paediatric associated neuropsychiatric disorders associate with streptococcus pyogenes)

Lab diagnosis 00:13:45

Lab diagnosis

Specimen – Throat swab, pus

Gram staining

- Gram positive cocci in chains

Culture

(5 – 10% CO2 at 370C)

- Blood agar
  - Pinpoint β hemolytic colonies

Liquid culture

- Granular turbidity is seen

Biochemical Test

- Catalase negative
- Bacitrein GAS – sensitivity

- Selective media
  - Crystal violet agar
  - PNF medium

- GBS – resistant

STREPTOCOCCUS PNEUMONIA

Streptococcus pneumonia 00:00:15

- Lanceolate shaped or flame shaped organism

Virulence factors 00:00:45

1) Capsular polysaccharide

↓

This inhibits phagocytosis

Demonstration reaction: Specific antisera are added along with methylene blue to the colonies

↓

Capsular swelling

2) C – substance

- C – RP test
  - β globulin produced in the liver is precipitate by antigen of pneumococcus
  - Acute inflammation causes elevation of CRP

3) Autolysin

- It is because it cleaves peptidoglycan which causes autolysis ‘

↓

Bile solubility & also responsible for Draughtman colonies

4) Pneumolysin

- Inhibits neutrophil chemotaxis

Clinical spectrum 00:04:48

- Lobar pneumonia
- Sub pneumonic effusion
- Otitis
- Pharyngitis
- Osteomyelitis
- Pericarditis
- Peritonitis
- Bacteraemia
- Septicaemia

Treatment

- Penicillin
- Resistance
  - PBP → PBP_2b

Epidemiology 00:06:20

a)Source of infection

- Carriers in URTI

b)Carriers

- 6 months to 5 years age group

c)Mode of spread – Droplet infection

d) High risk groups –

- Less than 2 years
- Post splenectomy
- Diabetes Mellitus
- Chronic lung disease
- Chronic liver disease

Lab diagnosis 00:07:50

I) Gram staining

- Lanceolate shaped or flame shaped

II) culture

- Blood agar - α hemolytic colonies (draughtman colonies or carrom coin colonies)
- Chocolate agar – Bleaching effect
- Liquid medium – uniform turbidity
- Bile solubility test – Bile increases autolysin activity

III) Bio chemicals

- Catalase – negative
- Optochin – sensitive
- Ferments insulin

Vaccination 00:11:35

PPV – 23	PCV – 13
• Usually indicated for age >65 years 19-64 years with indications • Indications i) CSF leak ii) Sickle cell anemia iii) Cochlear implant iv) DM v) Asplenia • Contraindications i) Pregnancy	• Indication – in less than 3 years • Can be given in adult with indication • Conjugate – CRM 197 ↑ C. diphtheriae ↑

ii) Malignancy iii) In children less than 3 years	Acts as adjuvant which increases the efficacy of vaccine • Schedule - 2,4,6 months & booster at 12-15 months

BRUCELLA

Brucella 00:00:10

- It is a zoonotic disease
- It is gram negative, non-sporing & non-capsulating organism
- It is a gram-negative coccobacillus
- Obligate intracellular organism → Reticuloendothelial system
- Noman classification
- 6 serovars

  1.B Mellitensis

  - Most pathogenic

  2.B Abortus

  3.B Bovis

  4.B Canis

  5.B Suis

  6.B Neotomal

Pathogenicity 00:02:26

- Mode of spread
  - Animals to man
  - Contact with tissues > milk & dairy products > inhalation
- Incubation period – 2 weeks to several months

Risk factor

- Occupational risk
  - Farmers
  - Shepards
  - Butchers
- Most commonly seen in Mediterranean areas, North America, south America. Australia;
- Brucella is also known as Malta fever; Mediterranean fever & undulated fever

---

Clinical manifestations: 00:04:52

I. Triad
   1. Fever with night sweats
   2. Hepatosplenomegaly
   3. Arthralgia / Arthritis

II. Typhoid like illness

III. CVS
   - Endocarditis
   - Aortic valve involvement

IV. CNS
   - Meningitis
   - Depression
   - Lethargy
   - Lymphocytic meningoencephalitis

V. Genitouringry
   - Salpingitis
   - Epidydimo-orchitis
   - Pyelonephritis

Lab diagnosis: 00:07:06

- Blood, bone marrow aspirate, CSF for culture
- Multiple blood culture should be taken (5-10ml for 3 days)
- Should be collected before antibiotic use
- Bone marrow comes positive and even if antibiotic is started
- Blood culture
  - Casteneda's method
  - BHI agar/broth → BA/CA → transparent non-hemolytic colonies
  - This method reduces contamination because it is directly sub cultured on the agar

Automated blood cultures

- Helps in early detection

Biochemicals

- Oxidase positive
- Catalase positive
- Serological diagnosis

SAT

↓

2-ME test

(2-Mercaptoethonal)

- Standard agglutination test
  - Gold standard

Procedure

Patient serum

↓

Added to killed suspension

↓

Titre – non-endemic area 1:160 (significant)

Endemic area

Paired serum

↓

Rising titres

Test in animals

1. Milk ring test
2. Rose Bengal test

Other tests

- PCR – Sensitive & specific
- Guinea pig inoculation

Guinea pig

↓

Inoculate in the thigh

↓

Spleen aspirate /LN aspirate

↓

Culture

Treatment 00:16:16

- Streptomycin + doxycycline
- Longer duration of treatment

Vaccine 00:16:44

- Live attenuated B abortus 19BA vaccine.

BORDETELLA PERTUSSIS

Bordetella pertussis 00:00:10

- Causes whooping cough / 100 days fever
- B pertussis – whooping cough
- B parapertusis – milder form of W.C
- B bronchuseptica – kennel cough and Dogs
- B avium – turkey

Virulence factors 00:01:46

1.Pertussis toxin

- It is specific for B pertussis
- It has 2 subunits

A subunit
It increases adenyl cyclase
(cholera vaccine)

B subunit
↓
Helps in binding

Role:

- causes hemagglutination
- ↑ isulin → hypoglycemia
- Damages tespiratony epithelium

2.Tracheal cytotoxin

- It is a part oof peptidoglycan of cell wall
- Affects the cilia of respiratory epithelium

3.Dermonecrotic toxin

4.Endotoxin

5.Adhesins

- Helps to bind to epithelial cells

Clinical manifestation 00:05:35

1.Catarrhal stage

- Most infectious
- Culture comes +ve
- Smear +ve

2.Paroxysmal stage

- Decreases in infectivity
- Culture -ve
- Smear -ve

3.Convalescent stage

- Defection of antibodies in serum

Epidemiology 00:07:25

- Source -A case of pertussis
- No carrier stages
- Age group affected - <5yers
- Spread → droplet infection

Lab diagnosis 00:08:45

- Symptoms – whoop which worsens in the night and also complaints of post – tussive vomiting

↓

Specimen in whoop – Nasopharyngeal aspirate (most important)

- 4-6 swabs at regular intervals
- Alginate swab → Culture

  - Dacron swabs for PCR

- Once smear is collected

  A. Culture smear – void coccobacilli arranged in thumbprint appearance

  B.Culture

  Regan Lowe culture   Bordet gengoci glycerin potato

  Mercury drops or bisected pearl appearance of colonies

  C.Detector of antibody

  - It is detection of IgA & IgM Seen in the later stage of disease

  D. Detection of antigen

  - Fluorescent tagged antibodies

  E.PCR

  - Most sensitive
  - Multiplex PCR
  - Eg- mycoplasma
    Adenovirus
    Infuezavirus

Transport Of Specimens

- Amies media
- Stuart media

Treatment 00:17:31

- Erythromycin ⎤
- Cotrimoxazole ⎦ 7-14 days
- For chemoprophylaxis – Erythromycin

Vaccination 00:18:26

A. Whole cell pertussis vaccine

- Given along with DPT
- Efficacy – 85%
- Heating & chemical inactivation of B pertussis bacilli
- Side effects

  a. Fever,

  b. Injection site pain

- Clinical infections

  a. AGE above 5yesr

  b. Child with neurological disorders

  c. Family with H/O epilepsy

B. Acellular vaccine

- Pertussis toxoid

  +

  Bacterial component – fimbria's
- Advantages
  - Decrease side effects
  - Administer in > 5years

PSEUDOMONAS AERUGINOSA

Pseudomonas aeruginosa 00:00:12

- Oxidase positive
- It produces non-lactose fermenting colonies on Macon key's agar (MAC)
- It is an example of pigment producing bacteria

Virulence factors 00:01:05

1) Alginate coat
    - Seen in mucoid strains of pseudomonas. It is responsible for biofilms
2) Colonisation
    - Mainly done with the help of pili, fimbriae as well as polar flagellum
3) Capsule
    - It helps in preventing phagocytosis
4) Multidrug resistance
    - It is because it contains genes encoding for drug resistance
5) Other factors

Virulence factors

- Tissue injury by toxins
  - Non – diffuse toxins
    - Type III secretion system
  - Diffuse toxins
    - Type II secretion system
    - Exotoxin A (most important virulent factor)
- Pigmentation
  - Pyocyanin
  - Pyoverdine – greenish – yellow
  - Pyorubin – red colour
  - Pyomelanin – brown-black in colour
  - Pyocyanin – blue to green
  - Produces by P. aeruginosa

Exotoxin A

↓

It inhibits

- Diphtheria toxin ⟵ proteins synthesis by inhibition of EF – 2

Clinical manifestations 00:07:30

1) Ventilator associated pneumonia (VAP)
2) Infections in chronic conditions
   - Cystic fibrosis – pseudomonas & S. Aureus
   - Bronchiectasis
   - 4 COPD
3) Infective endocarditis – most common in IV drug users
4) Bacteraemia

5) Ear infections
   - Swimmer's Ear
   - Malignant otitis media commonly seen in Diabetics
6) Eye infections – corneal ulcers
7) Soft tissue infections & Nail infections
   - Burns – most common organisms
   - Green nail – syndrome – Paronychia with green pus
   - Dermatitis
   - Ecthyma gangreosum
8) Others
   a) Cellulitis
   b) UTI catheterised patients
   c) meningitis

Lab diagnosis 00:12:00

- Sample Type – Pus, Wound Swab, Urine

Gram staining	Culture	Selective media	Motility
• GNB along • With pus cells	• Blood agas - β-hemolytic colonies • MAC – NLF colonies • Nutrient agar - Irregular colonies with metallic sheen - Pigment appreciation • King's media	• Cetrimide agar	• Motile • Polar flagellum

	- Enhances pigmentation		

Biochemicals

- Catalase positive
- Oxidase positive
- Indole positive
- Urease negative
- Citrate positive
- TSI – K/I with out gas & without H_2S

Odour

- Fruity odour O pseudomonas
- Fishy odour/seminal odour – proteus

AfraTafreen.com

HAEMOPHILLUS

H Ducreyi 00:00:10

- Causes STD – soft chancher or chanchroid
- Seen in developing countries
- Affects males > females → more common in heterosexual population

Symptoms 00:01:05

- Presents with painful genital ulcer which bleeds on touch
- Painful inguinal lymph nodes

Lab diagnosis 00:01:55

Gram staining
Pleomorphic
GN coccobacilli
↓
Bipolar staining
↓
Bacilli are arranged parallely
↓
School of fish or rail road track appearance

Cuture
Rabbit blood agar with isovitalex
↓
5% - 10% co2
A-8days

Catalase +ve Oxidase +ve

Treatment 00:03:16

- Azithromycin (1gm)

Haegypticus 00:03:38

- pleomorphic organism
- It is called Koch – weeks bacillus
- Clinical spectrum
  - Conjunctivitis
  - Brazilian purpuric fever

Differentiation of various species 00:04:40

Various species

Factor v	**Factor x**	**Factor v & x**
H parainfluenzas	H Ducreyi	H influenzas
H parahaemolyticus	H aegypticus	H haemolyticus
H paraaphrophilus	H aphrophilus	

H influenzae 00:07:00

- Oxidase +ve organism
- Pleomorphic gram-negative coccobacilli
- They require accessory growth factor – factor x & v
- It is capsulated

Important species 00:08:50

- H influenzae
- It causes meningitis (less than 2years)
- Others

  1.H Ducreyi – Chanchroid

2.H Aegypticus

3.H Parainfluenzas

4.H Aphorophrophilus

5.H paraaphrophilus

6.H Hemolyticus

7.H Parahemolyticus

H influenzas

- It is a fastidious organism

  ↓

  Requires accessory growth factor -factor x & v
- Blood agar – scanty growth

  ↓

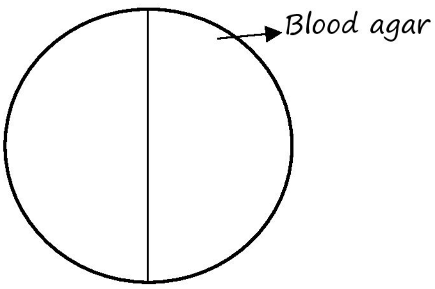
  BA has factor x but devoid of factor v

Chocolate agar

- Factor V is inactivated by NA Dase present in sheep blood
- Blood is added to molten agar at 75oc which inactivates NA Dase & it releases factor v from RBC's

Sattelitism

- S aureus is streaked perpendicular to H influenzae in streak line
- S aureus produces factor V



Virulence factor 00:15:40

Capsular polysaccharide (cp)

- Typing is done using cp & is divided Into 6 serotypes → a-f
- Most virulent – H influenzas serotype b
- Capsular antigen- PRP antigen (polyribosyl ribitol phosphate)

Clinical spectrum 00:18:05

1.CNS

- Pyogenic meningitis
- Age group - <2years
- Subdural effusion – hemiparesis and CNS Manifestation

2.Epiglottitis

- Acute airway obstruction caused due to cellulitis of epiglottis

3.Pneumonia

Non-typable streains 00:20:07

Causes

- Non – invasive conditions like
  - Otitis media
  - Sinusitis
  - Exacerbation of COPD
  - Pneumonia in adults

Lab diagnosis 00:21:00

1.Gram staining

- Pleomorphic coccobacilli

2.Culture

- Blood agar with staph aureus streak line
- Chocolate agar
- Levinthal's media
- H selective media

3.Quelleng's phenomenon

- CSF along with specific antiserum along with methylene blue

  ↓

  Demonstrate capsule

4.Latex agglutination test

Difference between various species of haemophiles

00:23:24

Treatment 00:25:16

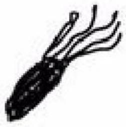
- Doc – cephalosporins
- For non-typeable strains – azithromycin
- For chemoprophylaxis – rifampicin

Vaccine 00:25:50

- PRP antigen → vaccine Hib vaccine

HELICOBACTOR PYLORI

- It is a curved gram-negative bacillus
- It is oxidase positive
- Oxidase positive organism
  a. Pseudomonas
  b. H. infulenzae
  c. H.pylori
  d. C. jejuni
  e. Aeromonas
  f. Plesiomonas
- It is catalase positive’
- It is Urease positive
  - Strong urease positive – proteus
- Helicobacter can colonise the mucosa of gastric epithelium
- Colonisation is facilitated by the following factors

Flagella ↓ Lophotrichous  Highly motile	Adhesins ↓ Adherence to the epithelium	Produces Amidase & Arginase Which helps in producing ammonia	Acid resistance ↓ Because it produces urease enzyme	Vacuolating cytotoxin ↓ Vacuole in the epithelium

			↓	

Urea → Ammonia + Co_2

↓	↓
Helps the organism from gastric acids	Produces neutral Environment

Clinical spectrum 00:05:08

a. Atrophic Gastritis

b. Acute Gastritis

c. Autoimmune gastritis

d. Pernicious Anemia

e. Peptic ulcer – 80% duodenal ulcer & 60% gastric ulcer

f. Adenocarcinoma

Acute gastritis

- Most common in antrum which can progress to duodenal ulcers
- H. pylori induces inflammation

↓

↓ Somatostatin

↓

↑ Gastrin

↓

Leading to food stimulated acid release

↓

Duodenal ulcer

Virulence factors 00:07:39

Cytotoxin Associated gene A	Molecular mimicry
	• LPS of H. pylori is identical to Lewis blood group antigen is present in parietal cells • Cross reactivity of antibody ↓ Symptoms occur

Lab diagnosis 00:09:12

- Invasive
  ↓
  Endoscopic biopsy is done
  - Histopathological examination
    ↓
    Starry – Warthin Staining
  - Microbiological examination
    - Staining
      G/S – curved GNB
    - ↓
      Culture
      - Skiraws media
      - Chocolate agar

        37°along with 10% Co_2
    - Biopsy urease test
- Non-invasive

- Biochemicals
  - Catalase +ve
  - Oxidase +ve
  - Urease +ve

Non – invasive

a. Rapid Urease	b. Stool Antigen test	c. Detection of antibodies
• Ask pt to take urea solution along with ^{13}c & blow in the tube ↓ Labeled Co_2 is produced and this is captured with mass spectrometry	• Follow – up of treatment	• Epidemiological purposes

Treatment: 00:14:15

- Triple therapy – OMC

O – Omeprazole
M – Metronidazole
C – Clarithromycin

7 – 14 days

↓

Test after 1 month

Rapid antigen test

↓

+ve

↓

Quadriple therapy

↓

O – Omeprazole

B – Bismuth subsalicylate

M – Metronidazole

T – Tetracycline

↓

Rapid stool test +ve

- Treatment based on antibiotic suspectibility

CAMPYLOBACTER JEJUNI

- It is a zoonotic disease
- It is curved, micro-aerophilic, gram – negative bacilli
- Catalase +ve & oxidase +ve
- It is isolated from intestine of animals – pets, cattles

  ↓

  Asymptomatic in animals
- Transmission
  - Most common – contaminated poultry
  - Unpasteurized milk
  - Direct contact with animals
  - Traveller's diarrhea
- Age group affected
  - Children
  - Elderly & immunocompromised
- Campylobacter species
  - C. Jejuni – Intestinal infections
  - C. Fetus – Extra – intestinal infections
  - C. Jejuni
  - C. Fetus

    Can be differentiated by growth @ 42°c
  - C. Jejuni – Growth at 42°C
  - C. fetus – No growth at 42°C

Virulence factors 00:04:06

Motility	Adhere to host tissues	Production of toxins	Capsule like ‘s’ layer which prevents complement mediated killing
• Single flagellum • Darting motility (also seen in V. cholerae)		• Enterotoxin – Heat labile – Resembles cholera toxin • Cytotoxin ↓ Cytolethal toxins	↓ C. Fetus

Clinical spectrum 00:07:20

Intestinal	Extra intestinal
• Inflammatory diarrhea – Blood stools – Fever – Pain Abdomen	• Fetus • Immunocompromised • Meningitis • Bacteria • Aneurysms • HLA 327 phenotype ↓ Reactive arthritis • GBS • Alpha chain disease

Lab diagnosis: 00:09:15

<table>
<tr><td rowspan="2">Gram Staining
• Gram – negative
• non- sporing
• ‘S’ shaped or gull wing shaped</td><td colspan="2">Culture</td></tr>
<tr><td>Transport medium
• Lary Blair medium</td><td>Culture
1. Skirrow’s media
2. Butzler’s selective media
3. Campy BAP selective medium
↑
Incubated at 37° in micro – aerophilic conditions
↓
Growth – Droplet like
Growth after 2-5 days</td></tr>
</table>

- Growth at 42°
  - C. Jejuni (thermorphilic)
  - C. Fetus (Non-thermophilic)
- Biochemicals
  - Catalase +ve
  - Oxidase +ve
- C. Jejuni subspecies
  a. C. Jejuni
  b. C. Doylei

- C. Jejuni is mainly identified by
  1. Nitrate test – +ve
  2. Hippurate hydrolysis – +ve
     Growth @ 42°c

Treatment: 00:13:30

- For diarrheal diseases
  - Azithromycin
  - Erythromycin
  - Ciprofloxacin
- Systemic – Inj. gentamicin

MYCOBACTERIUM TUBERCUOSIS-I

- Mycobacterium - non sporing, non - capsulated weakly gram positive and slightly curved bacilli

Classification 00:01:08

Mycobacterium

- M.TB
- MC

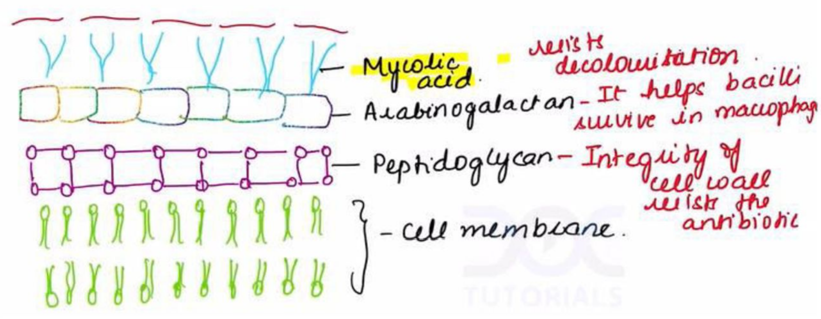
- M. leprae
- Hansen's disease

Non-TM
- MOTT
- Opportunistic infections

Saprophytic mycobacteria
- These are isolated from water, soil etc

Common properties

- Presence of mycolic acid→acid fastness integrity of cell well


Mycolic acid - resists decolourisation.
Arabinogalactan - It helps bacilli survive in macrophag
Peptidoglycan - Integrity of cell wall resists the antibiotic
- cell membrane.
TUTORIALS

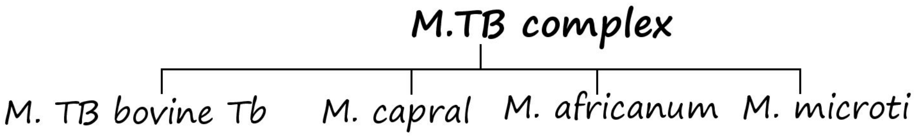
IMG 00:03:21

Antigen structure

- Cell wall antigen
- Cytoplasmic antigen

- Mycolic acid resits decolourisation

- Arabinogalactan -it helps the bacteria survive in macrophages
- Peptidoglycan- maintains integrity of cell wall and resists the antibiotic
- Cytoplasmic antigen-only used for testing for serodiagnosis
- M.TB complex


M.TB complex
M. TB bovine Tb
M. capral
M. africanum
M. microti

Risk factors 00:06:10

- Overcrowding
- HIV burden
- Smear positive→highly infectious
- If the number of bacilli is kore than 10^4 bacilli/ml
- Common is co-morbid conditions
- Women between 20-34 years

Clinical conditions 00:08:30

Pulmonary	**Extra-pulmonary**
• 80%	• 20% • TB lymphadenitis • Most common LN a) Supraclavicular b) Posterior crevicular • There is hematogenous spread of bacilli • TB of pleura – Pleural effusion – TB of upper airways including, larynx pharynx a7 epiglottis • TB of genitourinary tract • Renal TB • TB of endometrium, fallopian tube • TB of epididymitis • TB spine-cold abscess – Pott's spine • TB meningitis-children • TB pericarditis TB skin Scrofuloderma – Direct contact from skin Lupus volgaris – Apple jelly nodules

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Spread 00:13:05

- Contact with a case of TB
  - Droplet infection
- Contact with skin
- Unpasteurized milk
  - TB of GI tract
  - Most common site-caecum& terminal ileum

MYCOBACTERIUM TUBERCULOSIS-2

Lab diagnosis 00:01:12

- Pulmonary -sputum sample
- 2 samples

Spot sample

Early morning sample

- Sputum-contaminate with normal flora-Decontamination
  - Incraesed number of pus cells & hence secretions are thick-digestion
  - So there is decraesed chance of isolation of bacilli -concentraion
- 2 methods to decontaminate, digest & concentrate

Petroff's method
↓
Equal volume of sputum
+
4% NaOH
↓
Stabilize with 8% HCL

NACL+ 2% NaOH
↓
Liquefy sputum
↓
Increasing concentration of bacilli

Staining

ZN staining	Kinyoun's method	Auramine staining
• Primary stain - Carbol Fuchsin • Decolourization - H2SO4 • Counter stain - methylene - Pink - red bacilli in blue background	• Cold-staining • % of phenol in carbol fuchsin is increased • Increased duration of staining	• 1^o stain - auramine phenol • D.C - acid alcohol • Counter stain - potassium permanganate ↓ - Lower power - Green bacilli in a dark background

Culture

- It is gold standard
- A smear of ZN staining does not give the viability of an organsism
- Cultryee gives viability of organism

Solid medium	Liquid medium	Automated culture
• LJ medium	• Drugs susceptibility	a) MGIT
• Coagulated hen eggs	• 7H9 is used	b) BGCT/alert
• Mineral salts	• Sula	c) Bactec 460
		d) EPS system

- Aspergine
- Malachite green

  ↓

  Acts as a selective agent & inhibits the growth of normal flora
  - Dorset egg
  - Colony- rough, tough and buff colonies
  - Takes 4-6 weeks

- 7H9 & Sula are used in automated method

Advantages
- Continuous monitoring
- Detects early
- Drug susceptibility testing

- LJ medium-inspissation is done
- Culture can be positive if 10 - 100 bacilli are presented
- In smear to be positive - 10,000 bacilli/ml of sputum
- PCR(molecular method) - detects even single bacilli
- CBNAAT
  - 2 hours
  - It gives detects susceptability to Rifampicin (Genexpert)
- TB detection by PCR
- Quantitative PCR
- Extra pulmonary TB - paucibacillary

- CSF, synovial fluid, pericardial fluids, pleural fluid, aspirate-best CBNAAT

Latent TB 00:20:07

Mantoux

- To detect type IV hypersensitivity
- 0.1 ml PPD

↓

Flexor aspect of arm

↓

Erythema or induration

[48-72 hrs]

>10mm - positive

- 6-9mm - equivocal
- <5mm - negative

IGRA

(interoferon Gamma release assay)

↓

TB quantiferon gold

↓

A suspect patient

↓

Take sensitised T lymphoctes & expose it to ESAT6 / CFP10 antigen

↓

Inferons are released from T lymphocytes.

advantages- decrseaes false negative

Adults	**Children**
• Exposure to bacilli • Previous infection • False negative in - HIV - Severly immunocompromised state • False positive in	• Diagnostic marker

- BCG vaccination - NTM infection	

summary 00:24:41

- Screening -staining
- Gold standard-culture-post T/t culture is negative
- CBNAAT - 2-3 hrs
  - Extra-pulmonary cases
  - It tests for Rifampicin resistance
- PCR - detects even single bacilli
- Line probe assay-detection of resistance to antibiotics

Treatment 00:26:21

- Compliance of the patient
- 6 months

2 stages

Intensive	Continution
• 1st 2 months	• 2nd 4 months
• To render the patient to smear negative	• Dormant bacilli is killed + Prevention of relapse

DOTS→ to check compliance

- Drugs
  1) Rifampicin

2) Isoniazid
3) Ethambutol
4) Pyrazinamide
5) Strepromycin

- MDR - TB
  - Resistance to Rifampicin or isoniazid or any 1st line drugs
- XDR-TB
  - MDR - TB + resitance to fluroquinilones± resitance to anikacin
- CBNAAT - Rifampicin
- MGIT - drug susceptibility
- Line probe assay

Prophylaxis 00:30:42

BCG vaccine

- 0.1 ml → left deltoid
- Stain - Danish 1331 strain
- C/I
  - HIV positive
  - Severly immunocompromised state

Chemoprophylaxis

- 1 year - isoniazid

ATYPICAL MYCOBACTERIA

- Mycobacteria other than TB(MOTT) NTM
- Isolated from water, birds, soil etc.

Runyon's classification

1.Photochromogens - produce pigment in the light

2.Scotochromogen - produce pigment in the & dark

3.Non-photochromogen- do not produce pigments

4.Rapid grower - which grows in 1 week

Photochromogen 00:02:32

- M. marinum
  - Swimming pool granuloma or granuloma
  - Source-water
- M. Asiaticum
- M. simiase
  - It is isolated from monkeys
- M. Kansail
  - It produces orang yellow pigment on LJ medium at 37°C

Scotochromogens 00:04:04

- M. Scrofulaceum
  - Scrofula in children
- M. Gordonae
  - Isolated from tap water
- M. Szulgai
  - Scotochromogen at 37°C
  - Photochromogen at 25°C

Non - Photochromogen 00:05:41

- M. Avium complex
- M. Avium intracellular complex
  - Battey's baci
    - M. avium from birds cause infection decrease CO4 count
  - M. intracellular
- M. ulcer
  - Buruli ulcer
  - Painless ulcer caused by exotoxin
- M. Xenopi
  - Hospital out breaks
- M. para-TB
  - Affects cattle
  - Johnnie's Bacillus

Rapid growers-grows in 1 week

- M. smegmatus
- M. abscessus
- M. chelonae
- M. Fortutium

M. chelonae, M. Fortutium – Causes past injection abscess

Lab diagnosis 00:09:43

- ZN staining
  - Acid fast bacilli
- Catalase test
  - Strong positive
- Culture
  - LJ medium
    - Incubate in light
    - Dark

- Aryl sulphates test - positive for rapid growers

Treatment 00:11:29

- Erythromycin

  +

  Ethambutol

  +

  Rifampicin
- It is a prolonged treatment

ORGANISM NOT SEEN ON GRAM STAIN – RICKETTSIAE

FAMILY

Rickettsiaceae

Rickettsia
- Typhus fever group
- Spotted fever group

Orientia
- Scrub typhus

TYPHUS GROUP

Epidemic Typhus

- Organism – R. prowazekii
- Vector – Human louse
- Mode of transmission – Autoinoculation because of rubbing/scratching, Inhalation of feces.
- C/F – Acute febrile illness,
  ↓ myalgia and
  Headache
  Inc period 1-2 weeks
- Rash – 5th day – Rash on Trunk which later spreads to entire body

Epidemic Typhus

- R. typhi
- Rat flea, cat flea
- Inoculation or inhalation of feces of Rat flea
- Incubation period 1-2 weeks
  - Myalgia
  - Headache
  - Fever

- Diagnostic test- Weil-felix test

Spotted fever Group

Species	Disease	Vectors
R. rickettsii	Rocky mountain spotted fever	Tick
R. conorii	Indian tick Typhus	Tick
R. africae	African tick Bite fever	Tick
R.akari	Rickettsial pox	Mite

Rocky mountain spotted fever

- Vector – Tick
- Transmission – Bite of Tick
- Reservoir – Ticks, Dogs, Rodents
- Clinical features – Fever, Headache, rash, myalgia, Anorexia, Rash – appears first at the extremities, then trunk – maculopapular which later turns hemorrhagic

Complications: edema, pneumonitis, myocarditis DIC, Renal failure

- Associated with high mortality

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Indian Tick typhus fever

- Caused by R. conorii
- Vector – Tick
- Mode of Transmission – Tick Bite
- Clinical features – Fever, Anorexia, myalgia
- 50% cases have 'Eschar'

RICKETTSIAL POX

- Caused by R. akari
- Vector – Infected mite (found on surface of mice)
- Reservoir – Mice
- Clinical features – fever, headache, myalgia
- Rash – Vesicular Rash resembling chicken pox
- Eschar at inoculation site
- Regional Lymphadenopathy

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SCRUB TYPHUS (Chiggerosis)

- Caused by O. tsutsugamushi
- Vector – Trombiculid mites
- Clinical features: Triad
  - Rash (maculopapular)
  - Regional Lymphadenopathy
  - Eschar (site of Bite)
- Non– specific symptoms: Fever, myalgia, Anorexia
- Complications: Interstitial pneumonia and Encephalitis
- Epidemiology: Worldwide in distribution

Orgranism not seen on Gram Stain - Rickettsiae

Lab diagnosis

- Serological (Antibody detection)
  - **Non – specific**
    - Weil Felix
  - **Specific**
    - Latex Agglutination Test
    - CFT
    - ELISA
- Histopathological exam
  - Isolation with cell lines
- Animal inoculation Guinea pig
  - ↓
  - Neil – Mooses reaction (intraperitoneal inoculation in Guinea pig causes testicular inflammation)
  - ↓
  - Positive Tunica Reaction
- Molecular Method
  - PCR
  - OMP gene

Weil– Felix Test – Antigenic cross Reactivity

Principle: Heterophile Antigen test

- Patients with rickettsial infection produce antibodies that agglutinate certain non-motile species of protease.
- Such antibodies which react with these antigens are called heterophile antigens
  - Slide method
  - Tube method

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Results

Orgranism not seen on Gram Stain – Rickettsiae

Disease	OX - 19	OX - 2	OX - K
Epidemic Typhus	++++	+/−	−
Brill – Zinsser disease	Negative Or weakly Positive		
Endemic Typhus	++++	+/−	−
Rocky mountain spotted fever	++	++	−
Rickettsial pox	All are negative		
Scrub typhus	−	−	+++

Brill-Zinser's disease:

- Recrudescent illness in epidemic typhus
- Reactivation causes decreased immunity

Animal Inoculation

↓

Inoculate specimen intra peritoneally in Guinea pig

↓

Observe after 3-4 weeks

- R. rickettsia – scrotal necrosis
- R. prowazekii – only fever without testicular inflammation
- R. akari, R. typhi – fever with testicular inflammation (positive Tunica Reaction)

Molecular Method

- Whole Blood
- Blood clot
- Eschar

Acute stage

  - More sensitive than Blood
  - Positive even after T/t
- The organism or its genetic material is isolated from the eschar
- PCR
  - OMP gene
  - 16s rRNA gene

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ORGANISM NOT SEEN ON GRAM STAIN-SPIROCHAETES

Leptospirosis 00:17:00

- Caused by Leptospira
- Classification
  - L. interogens → Weil's disease
  - L. biflexa → saprophyte
- Made of transmission:
  - Indirect mode - water, surface contaminated with urine of animals.
  - Direct mode – Contact with urine/placenta of animals.
- Epidemiological determinants: 3r's
  - Rainfall (floods)
  - Rice fields
  - Rodents
- Clinical manifestations:
  - Mild anicteric febrile illness
    - 2 stages

      1. 1st stage → serum IgM absent
         - Septicemic stage
         - Symp – fever, myalgia, headache, conjunctional suffusion vomiting etc.

2.2nd stage: (Immune stage) →IgM elevated

- Symptoms- Meningitis, uveitis, optic neuritis peripheral neuropathy.

- In septicemic stage, it can be isolated from CSF & blood
- In immune stage, it can be isolated from urine
- Rx – Doxycycline – Best given in 1st stage
- Weil's disease -severe form of the disease
  - 2 stages
    - 1st stage
      I. septicemic stage
      II. lasts for 10 days
      III. High grade fever
      IV. Purpura / petechiae
      V. GI hemorrhages
      VI. Conjunctival hemorrhages
      VII. Can be isolated from CSF & blood
    - 2nd stage
      I. Immune stage
      II. Present with fever, jaundice, severe hemorrhages
      III. Lasts from 10 – 30 days
      IV. Isolated from urine
      V. In first stage, IgM levels are absent
      VI. In second stage, IgM is present
      VII. Septicemic stage, doxycycline is very effective.

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- Lab diagnosis:
  - Microscopy
    - Phase contrast or desk field microscopy
    - Coiled organisms with umbrella handle ends
    - Not commonly used
  - Animal inoculation – hamsters
  - Isolation
    - Not commonly done as it is a cumbersome process
    - If needed then
      - EMJH medium
      - Fletcher is medium
      - korthofs medium.
  - Serology
    - IgM levels ↑ from 1st week
    - IgM levels ↑ later – can be +ve for several years

Genus specific Antibody test	Serovar specific Antibody test
• Macro agglutination slide test	• Gold standard- Micro agglutination test (MAT)
• Microcapsule agglutination test	• Confirmatory diagnostic method
• Latex agglutination test	
• ELISA	
• Immuno chromatography test	

  - Molecular methods: - PCR – CSF sample
- Treatment
  - Doxycycline
    - DOC in early stages
    - Given for 7 days
  - Amoxycillin – sometimes given
- Chemoprophylaxis – Doxycycline

Clinical conditions	Causative agents
Relapsing fever	B. recurrentis
	B. duttonii
	B. hermsii
Lyme's disease	B. burgdorferi
Vincent's angina	B. vincentii

- Relapsing fever
  - Because of antigenic variation

↓

Febrile episode

↓

Followed by afebrile episode

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	Epidemic relapsing fever	Endemic relapsing fever
Caused by	B. recurrentis	B. hermsii
		B. duttonii
Transmitted by	Louse	Ticks
Natural host	man	Rodents
CNS manifestations	More common	Not seen
Hemorrhagic symptoms	More common	Not seen
Treatment	Doxycycline 1 single dose	Doxycycline 1 week

Lab diagnosis

- Microscopy - phase contrast or dark field microscope
- Animal isolation
- Antibody detection
  - IgM – elevated-early weeks
  - IgG - positive for several years
- Molecular methods
- PCR 16srRNA

Lyme's Disease 00:16:41

- Agent: B. burgdorferi
- Transmission: Ticks (Ixodes Ricinus)
- Clinical manifestation in 4 stages

1. 1st stage – erythema migrans (annular lesions)
2. 2nd stage – Dissemination
   - Skin lesions, joint pains
   - Banwarth's syndrome → Lymphocytic meningoradiculitis
3. 3rd stage
   - Persistent infection
   - Neurological sequalae
   - Encephalopathy arthritis
4. 4th stage
   - Post Lyme syndrome
   - Neurocognitive manifestations

- Lab diagnosis
  - Isolation → Blood or skin lesions and Kelley's medium
  - Microscopy – Dark field or phase contrast microscope
  - Ab detection – ELISA – IgM & IgM
  - Molecular methods – most sensitive & specific
- Treatment
  - Depends on presence or absence of CVS &CNS symptoms
  - If no CVS or CNS manifestations → Oral doxycycline
  - If CVS & CNS involvement is seen → ceftriaxone

Borrelia Vincentia 00:20:55

- Vincent's angina
- Commensal in mouth
- Presents with – greyish membrane that peels off easily

  resembles c- diphtheria
- Sometimes associated with other fusiform bacilli
- Treatment
  - Metronidazole
  - Penicillin

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SHIGELLA

- Classification based on serology & biochemical tests.
  1.A – S. dysenteriae- doesn't ferment mannitol
  2.B – S. Flexneri
  3.C – S. boydii
  4.D – S. sonnei
- Serotypes of S. dysenteriae
  - 15 serotypes of which 7 serotypes are important
  - 1. S. Shigae
    2. S. Schmitzi
    3. S. 3-7 serotypes
  - S. Shiga
    - Produces Shiga toxin
    - Mannitol is not fermented
    - Indole test-ve
    - Catalase test-ve
  - S. Schmitz – indole test +ve
- S. Flexneri
  - Antigenically more complex
  - 6 serotypes – 6th serotypes are the most important one
    - Indole test -ve
    - Based on produced gas from sugar, further divided into biotypes
      1. Manchester
      2. New castle
      3. Boyd 88.

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- Shigella boydii
  - Least common to cause dysentery
  - It consists of 19 serotypes
- S. sonnei
  - Antigenically homologous
  - Has only 1 serotype
- Pathogenicity
  - Mode of spread – 4‘Fs’
    1. Fingers (contaminated)
    2. Food
    3. Feces
    4. Fluid (water)
  - Infective dose – 10 -100 bacilli
  - Mechanism

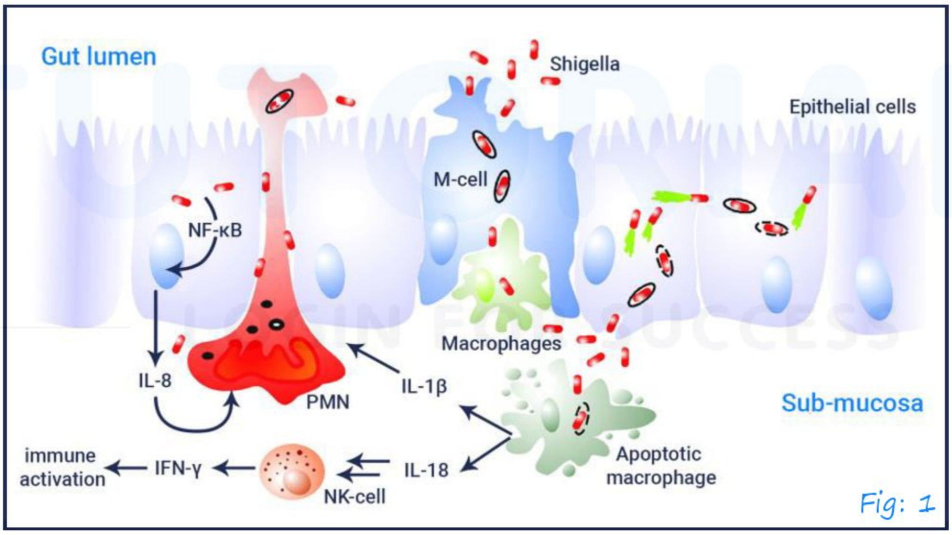
Gut lumen
Shigella
Epithelial cells
M-cell
NF-κB
Macrophages
IL-8
PMN
IL-1β
Sub-mucosa
Apoptotic macrophage
immune activation
IFN-γ
NK-cell
IL-18

Fig: 1

- Bacilli crosses the mucosa & enters sub-mucosa via 'M-cells'

↓

taking in by macrophages

↓

Apoptosis

↓

Bacteria are released

- Infected epithelial cells release cytokines (IL-Ibeta, IL-I8)

↓

Inflammation by recruitment of other inflammatory cells

↓

Colitis associated with shigella

- From affected epithelial cells, mucosa and adjacent cells are also affected

➢ Toxins

- Exotoxins

Shigella enterotoxin - I	Shigella enterotoxin -II
SHET-I	HET-II
Resemble cholera toxin	Associated with shigella Flexneri
Associated with shigella Flexneri 2a	

- Cytotoxin
  - Shiga toxin
  - Associated with shigella dystenteriae type -1
  - Resembles verocytotoxin of EHEC
- Endotoxin→causes inflammation & necrosis with ulceration.

➢ Clinical Features

- Incubation period
  - 2-4 days
  - Sometimes 1-2 days.
- Early phase
  - Diarrhea
  - Vomiting
  - Fever
  - Anorexia
- Phase of dysentery
  - Bloody stools with mucous
  - Severe abdominal cramps
- Phase of complications – most common in < 5 years of age
- Post infections phase – associated with $HLAB_{27}$ with S. flexeneri (3%)
  - After several years
    1. Reactive arthritis
    2. Ocular inflammation
    3. Urethritis

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- Phase of complications
  - Intestinal complications
    - Toxic megacolon
    - Perforation
    - Rectal prolapse
  - Metabolic complications – due to dysentery
    - Loss of Na – Hyponatremia
    - Loss of electrolytes
    - Hypoglycemia
    - Dehydration
  - Ekiri syndrome / Toxic encephalopathy→ due to metabolic complications
    - Seizures
    - Cerebral edema
    - Loss of consciousness
    - Delirium
  - Meningitis
  - Pneumonia
  - Kerato conjunctivitis
- Epidemiology
  - In developed countries – S. sonnei
  - In developing countries – s. flexneri
- Risk factor
  - Contamination
  - Overcrowding
  - Poor hygiene
  - Noncompliance to handwashing

TUTORIALS
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- Prevention – improving sanitation
- Lab diagnosis
  - Specimen – stool sample
  - Transport media
    - Cary Blair medium→ as it can isolate shigella, salmonella, vibriosis
    - Sachs buffered glycerol saline.
  - Enrichment media
    - To isolate bacilli from samples rich in commensals
    - Gram negative medium
    - Selenite F broth
    - Tetrathionate broth (inhibitory for some groups) – so not of importance when trying to isolate shigella
  - Selective media – MacConkey agar

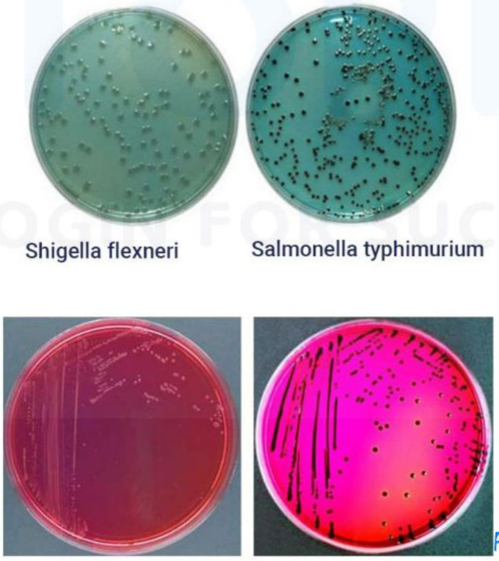

Fig: 2

TUTORIALS
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- Differential media
- Non – lactose fermenter colonies

  - S. sonnei – late lactose fermenter on prolonged incubation
  - Inhibitory because of presence of bile salts
- Hektoen enteric agar

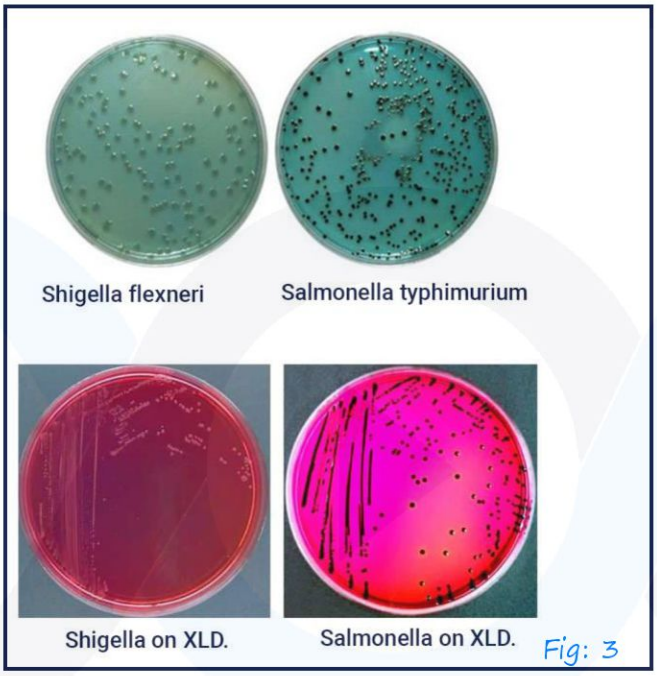
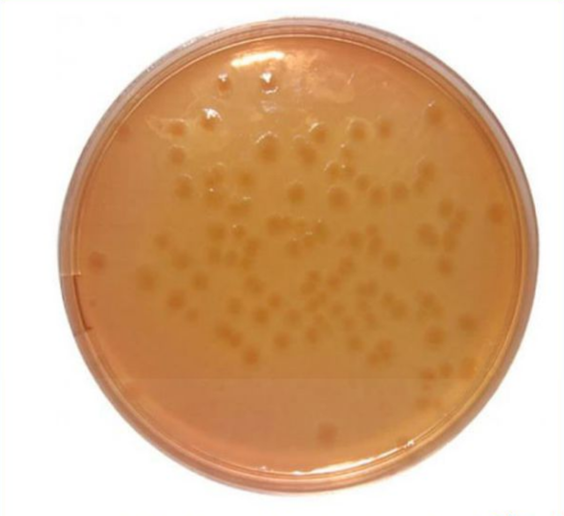

Shigella flexneri
Salmonella typhimurium
Shigella on XLD.
Salmonella on XLD.

Fig: 3

  - To isolate enteric pathogens
  - Produces green colored colonies in contrast to black centered colonies produced by salmonella.
- XLD
  - Commonly used for entheogens
  - Shigella produces pink pathogens
  - Salmonella produces pink colonies with black centers.
- DCA (Deoxycholate citrate Agar)
  - Pale, colorless colonies
  - Inhibitory of: – S. dysenteriae and Serotypes of S.flexneri

TUTORIALS
LOGIC FOR SUCCESS



DCA - (Deoxycholate citrate Agar) Fig: 4

- Motility –

  – non – motile

  – Gram -ve bacilli on gram staining.

➢ Treatment

- Ciprofloxacin – DOC
- Azithromycin, ceftriaxone – alternate drugs

➢ Prevention

- Improving sanitation
- Personal hygiene
- Hand washing
- Use of sodium hypochlorite for disinfection of stool.

➢ Biochemical tests:

- Catalase +ve - except S. dysenteriae type 1 and S. flexneri serotype 2a →Catalase -ve
- Oxidase -ve
- Mannitol fermentation– except S. dysenteriae and new castle biotype of S. flexeneri

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- Lactose and sucrose
  - non -fermenters
  - Except S. sonnei – late lactose & sucrose fermenter
- Anaerogenic – except S. flexneri biotypes – Manchester & Newcastle serotype 6.
- Indole
  - -ve -S. dysenteriae type 1
  - +ve – S. dysenteriae type 2
- Urease & citrate - -ve
- Triple sugar Iron (TSI) – K/A with no gas & H2s
- ONPG - -ve except S. sonnei
- Decarboxylase test - -ve except S. sonnei – decarboxylates ornithine

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GRAM NEGATIVE COCCI NEISSERIA

Gram negative cocci Neisseria

- Gram negative Cocci – Neisseria, Moraxella catarrhalis
- Genus, Neisseria – Gram negative diplococci

2 Pathogenic species

N. Meningitidis N. gonorrhea

- Virulence factors
  - Polysaccharide capsule – inhibits complement mediated phagocytosis
  - 13 serogroups of which 5 serogroups causes invasive disease (A, B, C, Y, w135)
  - Protease IgA
  - Outer membrane proteins

- Epidemiology
  - Season – winter
  - High prevalence – sub-Sahara
- Pattern –
- epidemic – A, w135
  - Outbreak – C
  - Hyperendemic – B
  - Sporadic – A, B, C, Y, wi35
- Risk factors
  - Travelers
  - Low socio-economic status
  - Hyposplenism

- Transmission
  - Droplet infection
  - Eventually by hematogenous route & olfactory nerve
- Clinical manifestations
  - Rash – 80%
  - Septicemia
  - Waterhouse Friedrichsen syndrome
  - Chronic meningococcemia
- Lab diagnosis
  - Specimen: CSF – transported immediately
  - Blood: transported & transferred to BHI broth and subculture on blood agar & chocolate agar
  - Nasopharyngeal swab – transport medium – Stuart's medium
  - Scrapings from rash – culture – New York city medium
  - Thayer martin medium
- CSF examination – divided into three portions

1. 1st portion – centrifuged
   - Supernatant
     - Capsular Ag detection – latex agglutination test
     - Biochemical analysis – increase protein, decrease glucose

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- Sediment
  - Gram staining
  - Gram negative
  - Lens shaped diplococci

2. 2nd portion – cultured on blood & chocolate agars- incubated with 10% Co2

↓

Come, tiny non - hemolytic colonies

3. 3rd portion – incubated in BHI broth

↓

Granular turbidity

↓

Sub cultured on blood agar & chocolate agar

- Biochemical tests
  - Oxidase positive
  - Catalase positive
- Serology – ELISA – capsular Ag
- Molecular diagnosis – PCR – Multiplex
  - Pyogenic meningitis
- Treatment: - DOC – 3rd gen cephalosporins
- Prevention
  - Chemoprophylaxis – rifampicin
  - Vaccine – bivalent – A, C
  - Quadrivalent – A, C, Y, w135
  - The serogroup not included in vaccination – B
  - The vaccine is given in 2 doses – 2-3 months apart

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  - Conjugate vaccine – an adjuvant is added (increase immunogenicity)
  - OMV based vaccine – has lipopolysaccharides
  - Protective for serotype B

N. Gonorrhea

- Gram negative Cocci
- Kidney shaped & non – capsulated
- Virulence factors
  - Fimbriae – prevents phagocytosis
  - Adhesion to host cells
  - Outer membrane protein
  - IgA protease
  - Transferrin binding protein
- Clinical features

Males	**Females**
• Acute urethritis • Incubation period – 2-7 days • Complications – Prostatitis – Balanitis – Edema of penis – Water can perineum	• Acute mucopurulent cervicitis • Salpingitis • PID • Fitz – high – Curtis syndrome

- In both sexes – ocular gonorrhea
  - Pharyngeal gonorrhea
- In pregnant women
  - PROM
  - Sepsis
  - Premature labour
- In neonates -Ophthalmia neonatorum
- Epidemiology
  - Source
    - Carrier
    - Symptomatic cases
  - Transmission
    - Sexual route
    - Vertical route
- Lab diagnosis
  - Specimen
    - Cervical swab
    - Urethral swab
  - Transport
    - Stuart's medium
    - Charcoal based Amie's medium
  - Microscopy – Gram negative kidney shaped intracellular diplococci

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- Selective medium
  - Thayer martin medium
    - Chocolate agar: + Vancomycin + Nystatin + colistin
    - Grey, opaque, Crenated colonies
  - New York medium
    - Lysed RBC's + T – Trimethoprim

      A– Amphotericin – B

      L – Lincomycin

      C – Colistin
  - Martin Lewis medium
- Biochemical tests
  - Oxidase positive
  - Catalase positive
  - Ferments glucose
- Treatment – 3rd gen Cephalosporin
- Prevention
  - Sex education
  - Barrier method of contraception
  - Contact tracing and treatment

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BACTERIOLOGY – VIBRIO CHOLERAE AND HALOPHILIC VIBRIO

Vibrio cholerae

- Strongly aerobic
- Motile organism – polar flagellum
- Oxidase positive
- Non sporing & non – capsulated
- Capsulated vibrio organisms
  - V. parahaemolyticus
  - V. vulnificus
- Salt – enhances its growth
- Habitat
  - Commonly isolated from marine environment
  - Sea water, sea food
  - Sewage

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Classification

- Based on salt requirement

1.Non – halophilic vibrio

- 1 % salt content required
- Do not grow on excess amount of salt
- E.g., – V. cholera V. minimus

2.Halophilic vibrio

- 7-10% salt content required
- Do not grow in absence of salt
- E.g., V. parahaemolyticus
- V. Vulnificus
- V. alginolyticus

- Heiberg classification
  - Based on fermentation of 3 sugar –
  - Mannose
    - Arabinose
    - Sucrose
  - I to VIII groups
  - V. Cholerae → group I
- Gardener's & Venkatraman's classification
  - Based on
    - Serotyping
    - Bio typing
    - Serogroups
    - Susceptibility to lytic phages *(phage typing)
  - Used commonly
- Sero grouping based on

  Somati 'O' antigen
- Groups
  - Vibrio
    - V. cholerae
    - Non-V. cholerae

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- Serogroups
  - Based on 'O' antigen
    - O1
    - O139
    - Non O1/O139
- Serotyping – based on small differences in antigen

  O1

  - Classical
  - Eltor

  Ogawa
  Ag: A, B
  Type – Mc

  Inaba
  A, C
  -2nd Mc

  Hikojima
  A, B, C
  Transition state

- Bio typing – based on biochemical reaction: susceptibility to polymyxin & lytic phages

  O1

  Classical
  -1-6 pandemics

  Eltor
  -7th pandemic

- Isolates that do not fit into either of the biotypes
  - Hybrid vibrio or Eltor variant
  - 2 variants
    - Matlab variant
    - Mozambique variant
- Phage typing – based on susceptibility to lytic phages

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- Pathogenesis
  - Mainly toxin mediated
  - Mode of transmission – contamination of food & water
  - Infective dose
    - 10^8 bacilli
    - Highly acid sensitive
  - Factors promoting its growth & transmission
    - Use of antiacids
    - Decrease gastric acidity
  - Pathogenesis
    - Crosses protective layer of mucus
  - Facilitated by motility by producing mucinase & proteolytic enzymes
  - Adheres to intestinal epithelium – via fimbriae type IV – toxin & colonization coregulated pilus (TCP)
  - Starts producing the cholera toxin

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- Enterotoxin

A — B – binding fragment

→ GM1 ganglioside

A → A1, A2

↓

Active peptide

Upregulates adenine cyclase

↓

Accumulation of CAMP

↓

Inhibits absorption of Na

↓

Causes accumulation of NaCl in lumen

↓

Water is lost to maintain osmolality

(passive loss of water)

TUTORIALS LOGIN FOR SUCCESS

Clinical features

- Watery diarrhea – rice water stools
  - Cloudy
  - Watery
  - Mucous flakes presence
  - No blood or pus
- rarely – vomiting's
- Fever is absent
- Muscle cramps – due to loss of electrolytes

Lab diagnosis

- In active cases – specimen is watery stools
- In carriers – rectal swab
- Transport medium – the bacteria do not multiply but keeps the organism viable

↓

Types:

1. Venkataraman Ramakrishnan medium
   - Contains crude salt with peptone & distill water
   - PH – 8.6-8.8
2. Alkaline salt transport medium
   - Crude salt, peptone, distill water, boric acid NaOH
   - PH – 9.2
3. Cary
   - Blair medium ‘advantage of also isolating shigella + Salmonella
   - PH – 8.4
4. Autoclaved sea water
   - Used when other transport medias are not available

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Direct microscopy

Gram staining

↓

Mucous

Flakes

- Curved gram-negative bacilli
- Parallel lines like fish in stream appearance

↓

'Fish in stream'

Motility

Darting or shooting star motility

Confirmation

Darting motility is inhibited when mixed with antisera

Culture

- Non – fastidious organism
- Grows better in presence of NaCl
- Incubation of 37°C
- Ordinary media

1.Nutrient agar – translucent colonies seen

2.Peptone water – uniform turbidity seen with surface pellicle

3.Blood agar – hemodigestion seen

↓

Non – specific lysis of RBC due to metabolic product of bacteria

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Incubation of commensals

1. Enrichment broth – Alkaline peptone water
2. Selective agar
   - Alkaline bile salt agar – oil drop colonies
   - Monsur's medium – black centers with halo colonies
   - TCB's – most common medium used
     - Yellow colored colonies
   - Mac con-key agar – pale colonies

↓ prolonged incubation

Late lactose fermenter (LLF)

Biochemical tests

- Oxidase – Positive (other organisms that are oxidase positive -pseudomonas, Plesiomonas, Aeromonas

Plesiomonas, Aeromonas – Decarboxylase test to differentiate

- Catalase - Positive
- Indole ring - Positive
- Nitrate reduction
  - Positive

Catalase, Indole ring, Nitrate reduction – Tested together called cholera red test – H_2so_4 + peptone with cholera

↓

Red colored nitroso indole ring

- Methyl red- Positive
- Urease - Negative
- Citrate – Proskauer test (VP-test)
  - Differentiates classical & Eltor
  - Positive for Eltor
  - Negative for classical

- Sugar fermentation tests
  - Glucose] fermented
  - Sucrose]
  - Mannitol
- String test –A colony is taken and emulsified with 0.5% sodium deoxycholate

↓ Turbidity test

Suspension becomes mucoid

↓ when tried to lift

String is informed

- Decarboxylase test: To differentiate
  - Vibrio – lysine
    - Ornithine
  - Aeromonas – Arginine

    plesiomonas– Lysine
    - Ornithine
    - Arginine
- Salt tolerance test
  - V. cholerae – up to 6% NaCl
  - Halophilic vibrio – 7 –10% NaCl
  - Doesn't survive without NaCl.

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Bio typing

Features	Classical	Eltor
• B- hemolysis of blood agar	Negative	Positive
• Chick embryo agglutination	Negative	Positive
• CAMP test	Negative	Positive
• VP test	Negative	Positive
• Polymyxin B	Susceptible	Resistant
• Group IV phage susceptibility	Resistant	Susceptible
• Cholera toxin gene	CTX1	CTX_2

Treatment

- Fluid replacement – main stay of treatment

  ↓

  ORS & IV fluids
- Antibiotics have limited role – as it is toxin mediated
  - Only in clearance of organism & ↓ fluids loss
- Tetracycline
  - Doxycycline
  - Azithromycin
- Chemoprophylaxis – tetracycline

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Prevention

- Hygiene
- Safe drinking water
- Early case detection
- Appropriate treatment to prevent outbreak
- Health education

Vaccines

- 2 types of oral vaccines

  1.Killed whole cell vaccine

    - Killed whole cell of v. cholera O1 (Classical and Eltor Ogawa & Inaba)
    - 2 doses → 7 days apart
    - Not given in < 2years.
    - In 2 - 5 years age → 3 doses
    - Given in areas considered to be a threat
    - Killed whole cell of V cholera o1 + recombinant cholera toxin B subunit

  2. oral live attenuated vaccine

    - Mutant strains that lack gene encoding for cholera toxin.

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Halophilic vibrio

- These resist higher salt concentrations ->6%
- Important halophilic vibrio – v. parahaemolyticus
  - V. vulnificus
  - V. alginolyticus

V. parahaemolyticus

- Virulence factors – presence of polysaccharide capsule
  - Hemolysin
  - Urease
- Clinical features
  - Acute food gastroenteritis –diarrhea →meat wash appearance of stool. or Dysentery
  - Abdominal cramps
  - H/O consumption of sea food
  - - undercooked or raw
  - Commonly oysters
- Can cause other infections
  - Wound infections
  - Otitis
  - Sepsis
- Lab diagnosis
  - Gram staining – bipolar staining
  - Motility – motile →peritrichous flagella and No darting motility.
- Culture → TCBS agar – sucrose non fermenter
  - Green color colonies
  - Blood agar – swarming is seen
  - Kanagawa phenomenon – In Wagatsuma agar (blood agar +salt)
  - Salt tolerance test – up to 8% salt content

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  - Urease test – positive
  - Treatment – self limiting
  - Tetracycline & doxycycline

V. vulnificus

- Most pathogenic halophilic bacterial vibrio
- Lactose fermenter
  - Only vibrio that ferments lactose
- Clinical features
  - In immune compromised, iron overload, liver dysfunction – sepsis
  - In immune competent – cellulitis with redness & necrosis

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v. alginolyticus

- Highly salt resistant
- Can survive at 10 % NaCl
- Clinical spectrum
  - Ear – otitis media
  - Otitis externa
  - Eye – conjunctivitis
  - Immune compromised – bacteria
  - Wound infections
- Treatment – Tetracycline – DOC
  - Surgical debridement in wound infections.

MYCOBACTERIUM LEPRAE

Mycobacterium labral

- Found less globally and a big burden in India
- Some important points:

(MNEMONIC)

Have I Cut Ample Loaf Of Bread?

H – Hansen's disease

I – ILRL bacillus (Difficult to grow in lab) – Grown on LG medium

C – Non – Cultivable – Artificial media

A – Animal model to cultivate

L – Long incubation period – (3-5 years); long generation time (12-13 days)

O – Obligate intracellular strictly aerobic organism

B – Bacilli which is acid fast requiring only 5% sulphuric acid for staining

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Have I cut ample loaf of bread Fig: 1

- Mode of transmission:
  - Nasal droplet infection – most common
  - Breast milk (vertical transmission)
- Communicability:
  - Very less
  - Required prolonged & intimate contact
- Incubation period - 3-5yrs is attributed to a long generation time of 12-13 days.
- Classification of leprosy: Ridley Jopling classification
  - Difference between lepromatous leprosy and tuberculoid leprosy
    - Spectrum of leprosy:

TT – BT ← BL →BL - LL

(Borderline tuberculoid) (Borderline) (Borderline leprosy)

↓ ↓

Characteristics	**Lepromatous leprosy**	**Tuberculoid leprosy**
• Bacillary bad	High/large	less
• Bacteriological index	3+	0-1+
• Cell mediated immunity (CMI)	Low	Normal
• Lepromin test	-ve	+ve
• (D4/CD8 cell ratio)	1:2	2:1 (Normal)

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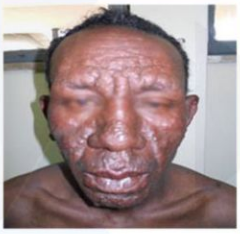
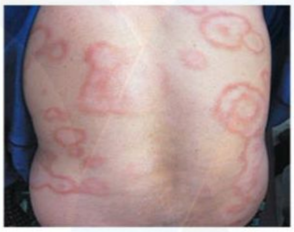
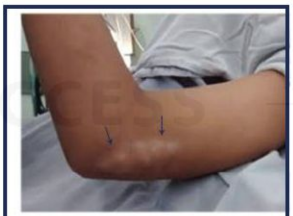
• Humoral immunity	Exaggerated	Normal
• Autoantibodies	Risk of such antibodies production	No risk
• Skin lesions	Symmetrical lesions- -nodular -asthma like plaques  Fig: 2	Asymmetrical – Hypopigmented patches with clearing in center  Fig: 3
• Nerve lesions	Late onset of nerve lesions/injury	Nerve involvement – Ulnar nerve -nerve abscess  Fig: 4

- Deformities
  - Leonine facies – lion like face
  - Wrist drop & foot drop
  - Claw hand
  - Loss of eyelashes & eyebrows.
- An exaggerated allergic response is seen in chronic course.
  - It is divided into

	Lepra Reaction type 1	**Lepra Reaction type II**
Hypersensitivity	Type IV	Type III
Th Response	TH 1	TH 2
Seen in	Borderline leprosy	Lepromatous leprosy
Manifestations	Inflammations of previous lesions, acute neuritis	Nodular lesion that are painful
Other organ involvement	Not involved	Testis, kidney & all other
Rx	Glucocorticoids	Glucocorticoids & thalidomide

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Lab diagnosis:

- Smear microscopy
  - ZN staining
  - Most important
  - Using 5% sulphuric acid

  - Acid fast bacilli are seen in clumps due to a lipid holding them together.
  - 'Cigar bundle appearance '
  - Present in foamy macrophages (Virchow cells)
- Cannot be grown on artificial media because it is an obligate intracellular organism.
- Animal inoculation
  - Foot pad of mice & Nine banded armadillo
- Antibody detection
  - ELISA – Not commonly used

Smears

- 6 sites are taken to prepare smears
  - Forehead
  - Cheek
  - Chin
  - Nasal mucosa
  - Ear lobe
  - Buttocks
- Slit skin smear
- Indices:

  A.Bacteriological index
  - Total no. of both dead & alive bacilli seen per oil immersion field.

  B.Morphological index
  - Important for post treatment
  - Uniform staining of bacilli is seen.
- Load is calculated based on this indices & also response to treatment can be detected.

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Tests to detect CMI

- Lepromin test
  - To detect delayed hypersensitivity and intact CMI.
  - Done by injecting 0.1 ml lepromin antigen (Intradermally on the forearm.)

Reading is taken at:

48 hours

- Early reading
- Fernandez reaction

↓

- Induration and erythema >10mm diameter

↓

- Exposure to lepra bacilli (doesn't necessarily mean active infection)

21 hours

- Late reading
- Mitsuda reaction

↓

- Nodule >5mm diameter

↓

Intact CMI

- This test helps in classification of leprosy lesions and helps in assessing prognosis of treatment.

Treatment

- Recommended drugs by clinical classification.
  - Dapsone
  - Rifampicin
  - Clofazimine

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Paucibacillary leprosy	**Multibacillary leprosy**
Lesions are <5	Lesions are >6
Smear negative	Smear positive
0 or 1 nerve involved	>2 nerves involved
T/t – Dapsone + Rifampicin (Once a month under supervision)	T/t – Dapsone + Rifampicin + Clofazimine
For 6 months ↓ Annual follow -up until 2 years.	For 1 year ↓ Annual follow for 5 years

- Chemoprophylaxis
  - Advised for close contacts especially for tuberculoid leprosy.
  - Dapsone – Drug of choice
  - Not recommended to everybody
  - As low communicability
  - Unless there is intimate contact
  - BCG vaccine
  - Arbitrary protection

GARDNERELLA VAGINALIS

- Part of normal female genital flora
- Bacterial vaginosis → outnumbers the normal count
- Morphology – pleomorphic
  - Small rods with metachromatic granules
- Culture – Hemolytic colonies → isolated best when incubated with 5% CO_2 in 24-48 hrs
- Bio chemical Reactions
  - Catalase
  - Urease
  - Indole
  - Oxidase

  Negative

Pre – disposing Factors

- Alteration in Normal vaginal flora
- Lactobacilli – maintain acidic PH

  ↓

  decrease in lactobacilli
- Pre term Labour
- Infections like chlamydia, HIV
- Vaginal douching

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Symptoms

- Greyish white discharge
- Dysuria
- No pruritis or inflammation

Lab Diagnosis

- Specimen- Vaginal discharge or swab
- Wet mount – 'Clue cells' → Vaginal epithelial cells adhered with

  +

  Bacteria coccobacilli
- PH > 4.5
- Whiff Test – Vaginal discharge + 1 % KOH

  ↓

  Fishy Odor

AMSEL'S CRITERIA

- ④ - 3 POSITIVE
- Thin profuse discharge adherent to the vaginal wall
- Ph > 4.5
- Fishy Odor
- Clue cells

Gram staining: Nugent's scoring

- Score ≥ 7 (significant)

Treatment – DOC – Metronidazole X 7 days

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RAT BITE FEVER

- Septic fever
- Painful polyarthritis
- Petechial rash

C/F

- 2 Pathogens
  - Spirillum minus
  - Streptobacillus moniliformis
- Present as normal flora in the throat of rats
- Mode of spread
  - Bite of rat
  - Water or milk contaminated with rat's saliva

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Agents →	S. moniliformis ↓ Streptobacillary rat fever	S. minus Sudoku (Japan)
Incubation period	7 – 10 days	3 weeks
C/F	• Fever • Polyarthritis • Rash	• Fever • Polyarthritis • Rash • Lymph node enlargement
Motility	Non - motile	Motile
Culture	Cultivable	Non - cultivable

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DOC: - Penicillin

- Streptomycin
- Doxycycline

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PASTEURELLA MULTOCIDA

- Isolated from respiratory tract of animals
- Mode of spread – Animal bite
- C/M
    - Bite site: Inflamed and Swollen with regional lymphadenopathy
    - Meningitis
    - Bacteremia
    - Chronic respiratory infection

Lab diagnosis

- Gram staining – G.N. Coccobacilli with bipolar staining
- Oxidase positive
- Indole positive
- Non – fastidious organism – Nutrient agar at 37°c

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LEGIONELLA PNEUMOPHILA

Morphology

- Fastidious
- Gram Negative
- Pleomorphic bacilli

Classification: 19 species – Human disease

↓

L. pneumophila

Epidemiology

- Reservoir – aquatic bodies
- No human-to-human Transmission
- Artificial Bodies- Harbour bacteria

Clinical syndromes

- Pontiac fever
- Legionnaire's disease (atypical pneumonia)

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Transmission

- Most common oropharyngeal colonization, contaminated drinking water
- Aerosol – hospital setting
- Manipulation of respiratory Tract

Pre – disposing factor

- Smoking
- COPD
- HIV – AIDS
- Surgery ↓ GA

Pathogenesis

Legionella enters the lungs

↓

Attachment to respiratory mucosa by type IV pili, OMP's

↓

Neutrophils and Macrophages are Recruited to site

↓

coiling phagocytosis → Alveolar macrophage phagocytose Legionella

↓

Evades killing by inhibiting phagosome-lysosome fusion

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Clinical Features	
Pontiac fever	**Legionnaire's disease**
• Flu – like illness with fever, Headache, malaise • Self-limiting • Incubation period :1 -2 days	Interstitial Atypical pneumonia **Symptoms** • Non – productive cough • Fever • Dyspnea • Chest pain **Extrapulmonary** • Blood borne dissemination • Myocarditis • Pericarditis • Prosthetic valve endocarditis

Lab diagnosis

- Samples- Sputum, BAL, Bronchial wash

Lab diagnosis

Gram staining

- Poorly stained coccobacilli
- Plenty of neutrophils

Culture

BCYE Agar

↓

Buffered charcoal yeast extract

↓

5% CO_2 for 3 – 5 days

↓

Ground – glass appearance

Biochemical Reaction

- Catalase Test – Positive
- Hippurate Hydrolysis – Positive
- Oxidase – Negative

Antibody detection

- Done for epidemiological purpose
- 12 weeks – detected late
- Limitations – Cross Reactive

Urinary Antigen Test

- Detected from 3 days of onset – 2 months
- L. pneumophila serogroup 1 Antigen
- Positive even after use of Antibiotics

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Molecular Methods – To isolate from water bodies

Prevention

- Routine culture of hospital water source
- Disinfection of water

Treatment

- Azithromycin
- Quinolones

ERYSIPELOTHRIX RHUSIOPATHIAE

- Isolated from animals like fishes, turkeys
- Causes erysipelas in animals
- Animal handlers, butchers – occupational risk
- Erysipeloids → violaceous swelling without pus but is very painful
  - Whale finger or Seal finger

Lab diagnosis

- GRAM STAINING – Gram positive bacilli in pairs, singles or chains
- Culture – BA – α Hemolytic colonies
- Biochemical
  - Catalase – Neg
  - Oxidase – Neg
  - Indole – Neg
  - TSI – produce H_2S

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Treatment

- Sensitive to penicillin
- Intrinsically resistant to vancomycin

HACEK AGENTS

- Commensals in oral cavity
- Gram negative coccobacilli
- Capnophillic
- 3% of total endocarditis
- Seen in valvular disease or patients who undergo dental procedure

H	A	C	E	K
(Haemophilus)	(Aggregatibacter)	(Cardio bacterium hominis)	(Eikenella corrodens)	(Kingella Kingae)

CA HIA

- H. parainfluenza – factor V only
- H. aphrophilus
- H. paraphrophilus – factor X only

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Aggregatibacter: Most common member to cause endocarditis

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Cardio bacterium:

- Large vegetations in endocarditis
- Non- motile, pleomorphic
- Gram staining – Tear drop or dumbbell shaped colonies

Eikenella corrodens

- No flagella
- Motile with twitchy movement – Type IV pili
- Capsulated, Gram negative bacilli

Kingella kingae

- Causative organism – osteoarticular lesions in pediatric age group
- Endocarditis
- Age group – 6 months – 4 years
- Plump gram-negative coccobacilli

Treatment

- Ceftriaxone – DOC x 4 week
- Eikinella – Ampicillin

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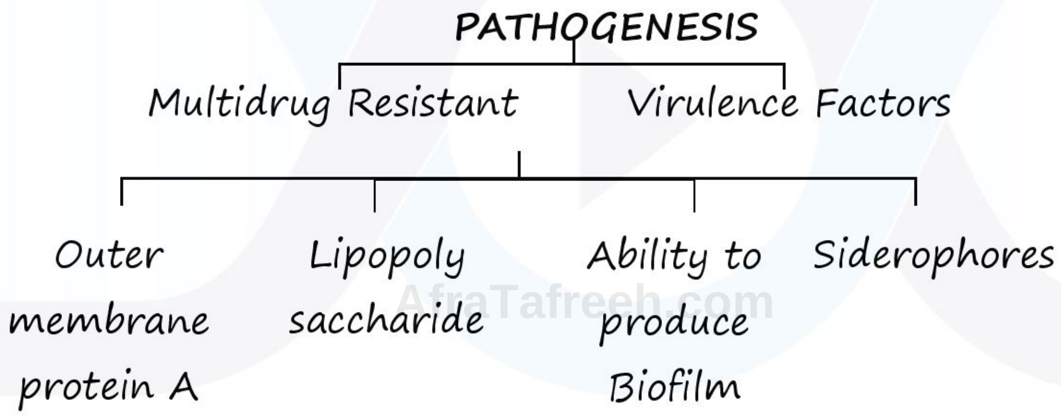
ACINETOBACTER BAUMANNII

- Saprophyte
- Nosocomial pathogen
- Source
  - Heavily contaminated hospital environment
  - Unhygienic practices of hospital staff
  - Commensals on skin, oral cavity etc
  - Affects immunocompromised patients.


PATHOGENESIS
Multidrug Resistant
Virulence Factors
Outer membrane protein A
Lipopoly saccharide
Ability to produce Biofilm
Siderophores

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Clinical spectrum

- VAP (Ventilator associated pneumonia)
- CLABSI (Central line associated blood stream infections)
- CAUTI (Cather associated UTI)
- Post – neurological meningitis
- Burns
- Skin & soft tissue infection
- Septicemia

LAB DIAGNOSIS

- Obligate aerobe
- BA – non – hemolytic colonies
- MAC – NLF colonies / pale color colonies
- Bio chemical Tests: Catalase positive

  - Oxidase Negative

  - TSI – k/k with no gas & no H_2S
- Gram staining – Gram negative coccobacilli

TUTORIALS
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FRANCISELLA TULARENSIS

- 'Tularemia'– Plague – like illness
- Zoonotic disease
- Highly infectious – category A of Bioterrorism
- Source – Animals - Animals carriers
- 3 modes of spread
  - Contact with wild animal
  - Aerosols
  - Contaminated food and water

Clinical Manifestations		
Ulceroglandular tularemia ↓ Ulcerative lesion at site of inoculation + Lymphadenopathy	Pulmonary Tularemia • Lab workers • Atypical pneumonia	Oropharyngeal Tularemia • Due to consumption of under cooked meat • Pharyngitis + Cervical Lymphadenopathy

AfroTafreeh.com

TUTORIALS LOGIN FOR SUCCESS

Oculoglandular Tularemia	Typhoid – like illness
Conjunctivitis + Periauricular Lymphadenopathy	Highly infectious

Culture – highly fastidious – culture is difficult

Selective Media	
BCG medium (Blood cysteine glucose agar) ↓ α hemolytic & grey colonies	CHAB agar (Cysteine heart agar + 9% sheep Blood)

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Handling: BSL III

Biochemical Reaction – Catalase – weakly positive – Oxidase – negative

Gram staining: Coccobacilli with Bipolar staining

Mainstay: Antibody Detection

DOC: Gentamycin

IMMUNOLOGY

Introduction to immunology, Innate & acquired immunity part – 1

Innate immunity 00:00:32

- Immunity – resistance offered by the host to any foreign substance or micro – organisms
- Immunity
  - Innate immunity
    - Immunity from birth
    - Constitutional make up
  - Acquired immunity
    - Immunity that is acquired during course of life
- Innate
  - Species specific immunity
  - Racial immunity
  - Individual immunity
- Innate immunity
  - Age
  - Hormones
  - Nutrition – malnutrition
- Innate immunity
  - Present from birth
  - First line of defence
  - Prior exposure to antigen is not needed

- It is not specific
- It does not bind to any specific binding site on the host cell
- It is less diverse
- Immunological memory is absent

Interaction 00:06:40

Receptor interaction

MAMP's
Microbial
associated
Molecular patterns
↓ Involves
LPS
Teichoic acid
flagella

PRR

- Pathogen/pattern recognizing receptor

↓

Present on host cell

↓

- Toll – like receptors
  - 13 types are present
  - Non – specific receptors

↓

- It tends to stimulate genes coding for cytokines

↓

- Release of cytokines

↓

- Anti – microbial activity

Acquired immunity 00:10:25

- Acquired during course of life
- More specific
- Response is slow & sluggish but highly specific
- Antigen specific action
- T cells, B cells classical pathway
- Immunological memory → memory cells are involved

2 types of cells

Effector cells   Memory cells

↓

On subsequent exposure

there is quicker reaction

↓

Recognizes the antigen

AiraTaireen.com

Factors that are involved 00:13:57

Immunity	**Acquired immunity**
• Physiological barriers • Anatomical barriers • NK cells • Complement – Alternate pathway – Mannose binding pathway • Alternate pathway is	• T – cells • B – cells • Classical compliment pathway

activated by endotoxin • In Mannose binding pathway, Mannose carbohydrate radius activates it • Dendritic cells Releases cytokines directly ↓ Responsible for inflammation APC ↓ Antigenic peptide to T cell receptor • Acute phase protein – C- RP	

INTRODUCTION TO IMMUNOLOGY, INNATES & ACQUIRED IMMUNITY PART – 2

Components of innate immunity 00:00:14

a) Anatomical barrier – skin →physical inhibition

- Produces sebum

  ↓

  Inhibit organism growth

b) Physiological barrier – temp

- PH – ↓PH – inhibit growth of microbes
- Cilia in lower respiratory tract

  ↓

  Inhibit entry of organisms
- Gastric mucosa →PH ↓ – gastric acid
- Lysozyme – Tears →antibacterial
- Spermine – semen →Inhibits GP organisms
- Enzymes

c) Macrophages

- In innate immunity →produces cytokines
- Antigen – presenting cells →acquired immunity

d) Cytokines

e) NK cells – Act on tumour cells & also on virus infected cells

f) Most cells → present in respiratory mucosa

- IgE antibody mediated →Histamine PG's

  ↓

  Killing of organisms

g) Complement pathway

- Alternate complement pathway → Endotoxin
- Mannos binding pathway → Mannose carbohydrate's residue

h)Fever

i) Acute phase proteins (APP):

- Synthesised by liver
- Inflammation – ↑ or ↓ of AP proteins

Positive APP ↑ inflammation	**Negative APP ↓**
• Serum amyloid A • Coagulation proteins • Complement proteins • C- RP	• Transferrin • Antithrombin

- APP are also synthesised by adipocytes, endothelial cells & fibroblasts

C- Reactive protein 00:09:45

- It is participated by C- Carbohydrates of pneumococcus
- Normal 0.2mg/dl
  - Increase from 0.2 – 1mg/dl (mild) due to cold, pregnancy

  - Moderate increase due to MI, pancreatitis, Bronchitis
  - Marked increase due to major trauma, vasculitis
- Hs – CRP – diagnosis MI
  - Test – CRP –
    - latex agglutination test
    - Nephelometry
    - Turbidometry

IMMUNOLOGY

Antigen 00:00:13

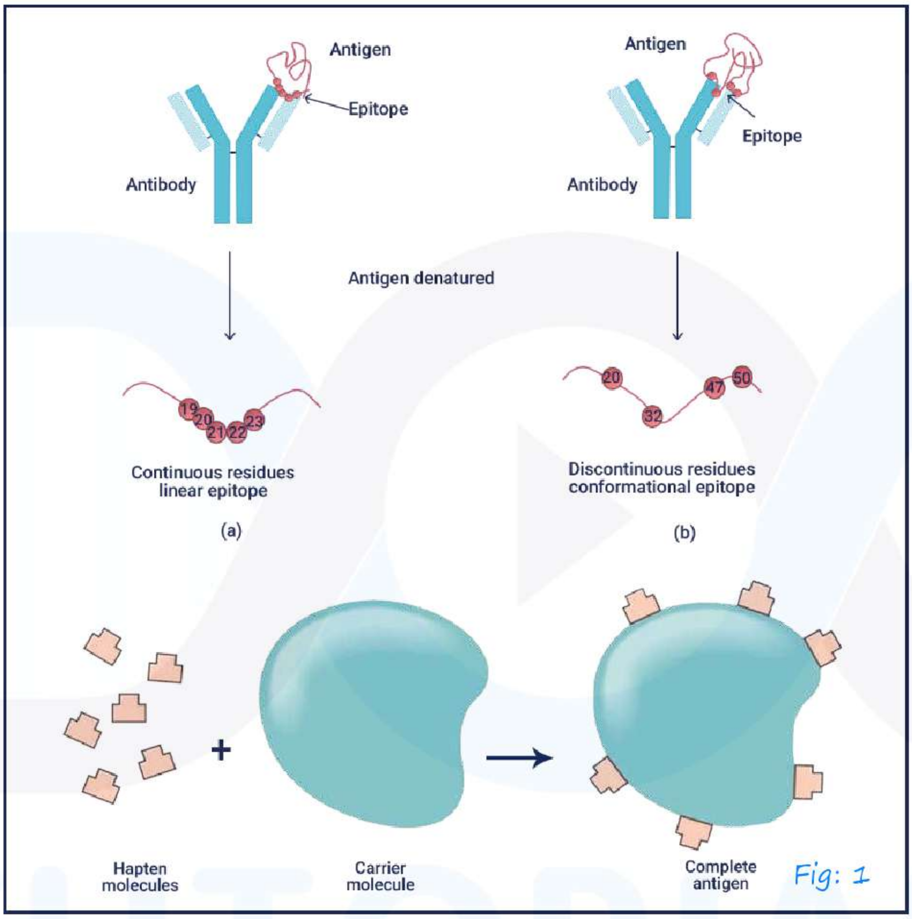
Antigen
Epitope
Antibody
Antigen
Epitope
Antibody
Antigen denatured
19
20
21
22
23
Continuous residues
linear epitope
(a)
20
32
47
50
Discontinuous residues
conformational epitope
(b)
+
Hapten
molecules
Carrier
molecule
Complete
antigen

Fig: 1

- A foreign substance or molecule that is capable of mounting an immune response in the form B cells & T cells

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- The 2 properties of an antigen
  - Antigenicity
    - Ability to specifically bind to the products of the two responses
  - Immunogenicity
    - Ability to mount immune response
      - B cells
        - Effector plasma cells
        - Memory cells
      - T cells
        - Effector plasma cells
        - Memory cells

Types 00:02:18

- Linear
- Conformational

Hapten 00:02:35

- A low molecular weight molecule

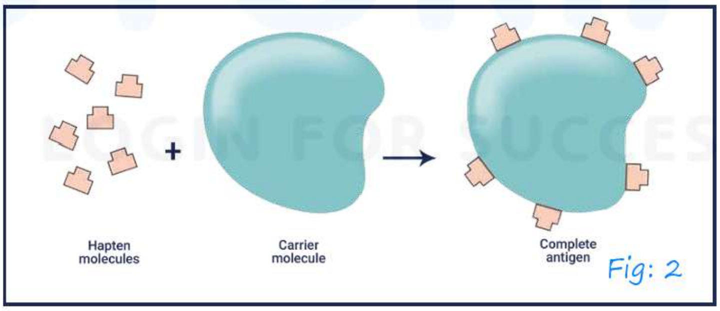
Hapten molecules
+
Carrier molecule
Complete antigen

Fig: 2

- Lacks immunogenicity

  ↓

  So, no immune response
- Antigenicity is present

- Hapten + carrier (protein) → complete antigen

  e.g., contact dermatitis

  Nickle (Hapten) + skin protein → immune reaction
- Types
  - Simple – single epitope
  - Complex – 2 or more epitope

Epitope 00:04:53

- Smallest unit of antigenicity
- Paratope
  - Present on the antibody which interacts with corresponding epitope of a specific antigen

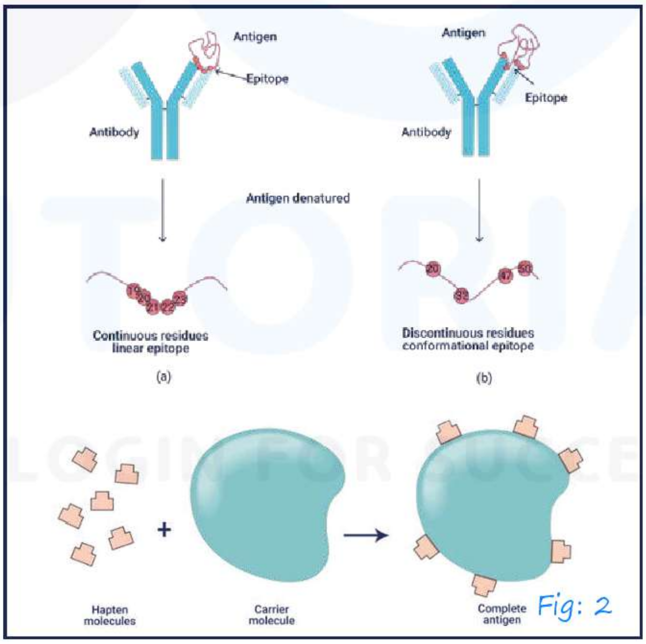
Antigen
Epitope
Antibody
Antigen
Epitope
Antibody
Antigen denatured
19
20
21
22
23
20
32
47
50
Continuous residues linear epitope
(a)
Discontinuous residues conformational epitope
(b)
+
Hapten molecules
Carrier molecule
Complete antigen

Fig: 2

Antigen – Host relationship 00:07:48

- Types of antigens based on antigen – Host relationship
  - Self – antigens
    - Does not mount immune response
    - Belongs to host
    - Immunological tolerance
      ↓
      Autoimmune diseases
  - Foreign – antigens
    - Doesn't belong to host
    - Types
      - Alloantigen ↓ Species specific
      - Iso antigen ↓ Subset of species
      - Hetero antigen ↓ Different Species

Heterophile antigens 00:09:59

- Type of hetero antigen
- Antibodies are produced against different antigens of different species that share the same epitopes
- Significant in diagnostic tests
- 3 important diagnosis tests based on this
- 1- Weil Felix test
  - For diagnosing typhus fever
  - Detects antibodies against Rickettsia antigen with cross reacting protein antigen
- 2- Paul Bunnel test
  - For diagnosing infectious mononucleosis
  - Sheep RBC' s is used to detect antibodies against EBV

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- 3- Streptococcal NGI cold agglutination test
  - For diagnosing atypical pneumonia
  - Detects antibodies against M. pneumonia with across reacting antigen of Human 'O' RBC' s and streptococcal MG antigen

Factor that effects immunogenicity 00:13:35

1.Size of antigen

- Larger the size – better immune response

2.Structural configuration

- Homopolymer with less amino acids – poor immune response
- Complex structure with multiple amino acids– better immune response

3.Chemical nature

- Order of immunogenicity
- Protein > carbohydrates > lipid> nucleic acid

4.Susceptibility to tissue enzymes

- When an antigen is broken down into multiple fragments, all its epitopes are exposed

5.Genetic factors

Immune response is different even with same species

Fast responders	Slow responders	Non – responders
• Produces antibodies quickly	• Slow antibody production and requires multiple stimuli by the antigen	• No response despite multiple stimuli

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6.Foreigners

- Further it is in terms of phylogenetic classification, better is the immune response
- So, in Humans, plant antigens mount a higher response compared to animal antigens

7.Optimum dosage → immune response

- If too less or more → no response by the immune system
- If more → immunological paralysis

8.Route of administration

- Parental – best response – maximum response

9.Repeat dose of antigen

- Essential to develop memory cells

  ↓

  Important in vaccination

10. Effect of prior administration of antibody to that specific antigen – low response

11. Multiple antigens

- Response can be different from person to person

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Adjuvants 00:20:25

- Substances that are added to antigens to increase the immunogenicity
- Importance – vaccine production
- Mechanism

  1.Delay the release of antigen

↓

Antigen binds to adjuvant

↓

Precipitation

↓

Slowly released from sight of inoculation

2.Granuloma formation

↓

Due to presence of activated macrophages

T helper cells activation

3. Activated TH cells

↓

Releases cytokines that act on B cells

↓

Better immune response

4.Activating phagocytosis

↓

Ag + adjuvant → phagocytosis

e.g., Adjuvants

alum, mineral oil

Freund's complete & incomplete adjuvant

Lipopolysaccharide gram negative bacilli → B. pertussis

↖ ↓

Adjuvant DⓅT vaccine

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TYPE 1 – HYPERSENSITIVITY REACTION

Hypersensitivity 00:00:11

- Fatal response or an injurious response to an allergen in an already sensitized individual.
- Two types:

  1.Immediate

  - Within minutes to hours
  - Mediated by antibody (IgG/IgM/IgE)

  2.Delayed

  - Within weeks
  - Mediated by T-cells. (T_{DTH})

Hypersensitivity types and them mechanisms 00:02:15

	Hypersensitivity Types and Their Mechanisms			
	Type 1	Type II	Type III	Type IV
Immune reactant	IgE	IgG or IgM	IgG and IgM	T cells
Antigen form	Soluble antigen	Cell-bound antigen	Soluble antigen	Soluble or cell-bound antigen
Mechanism of activation	Allergen-specific IgE antibodies bind to mast cells via their Fc receptor. When the specific allergen binds to the IgE,	IgG or IgM antibody binds to cellular antigen, leading to complement activation and cell lysis. IgG can also mediate ADCC	Antigen-antibody complexes are deposited in tissues. Complement activation provides inflammatory mediators and recruits neutrophils. Enzymes	T_H1 cells secrete cytokines, which activate macrophages and cytotoxic T cells.

	cross-linking of IgE induces degranulation of mast cells.	with cytotoxic T cells, natural killer cells, macrophages, and neutrophils.	released from neutrophils damage tissue	
Examples of hypersensitivity reactions	Local and systemic anaphylaxis, seasonal hay fever, food allergies, and drug allergies	Red blood cell destruction after transfusion with mismatched blood types or during hemolytic disease of the newborn.	Post-streptococcal glomerulonephritis, rheumatoid arthritis. and systemic lupus erythematosus	Contact dermatitis, type I diabetes mellitus, and multiple sclerosis

Type-1 Hypersensitivity 00:05:42

- Mechanism occurs in 2 phases:

1.Sensitization phase

– Allergen exposed for 1st time

↓

Antibody's (IgE) Fc part binds to Fc receptors on mast cells

↓

Sensitized mast cells (mast cells coated with IgE)

- This first interaction with allergen is called priming dose
- primary dose in most effective when allergen's route of administration is parenteral

2. Effector phase

allergen is exposed subsequently

↓

Binds to fab portion of IgE

↓

Activation of mast cells

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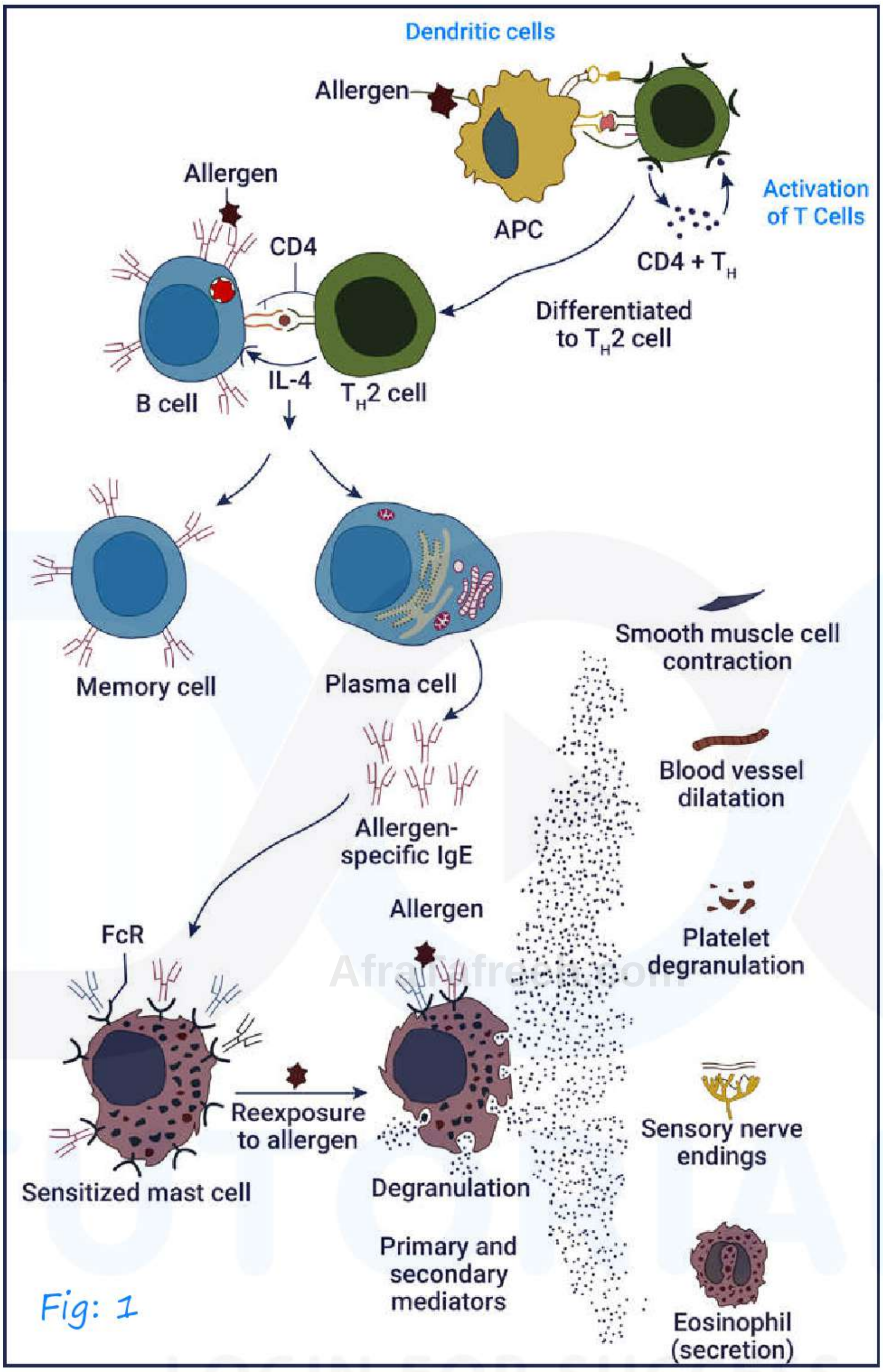
Dendritic cells
Allergen
APC
Activation of T Cells
$CD4 + T_H$
Allergen
CD4
Differentiated to T_H2 cell
B cell
IL-4
T_H2 cell
Memory cell
Plasma cell
Smooth muscle cell contraction
Allergen-specific IgE
Blood vessel dilatation
FcR
Allergen
Platelet degranulation
Reexposure to allergen
Sensitized mast cell
Degranulation
Sensory nerve endings
Primary and secondary mediators
Eosinophil (secretion)

Fig: 1

- Sensitized mast cells release 1° &2° mediators after degranulation that results in -smooth muscle cell contraction
  - Blood vessel dilation
  - Platelet degranulation

Primary mediators 1°	Secondary mediators 2°
• Preformed • Present in mast cells • Released on contact with allergen	• Formed after contact and then released • L- leukotriene • P- prostaglandins • C- cytokines • B- Bradykinins
• H- Histamine, Heparin • S- Serotonin • P- proteases • E- Eosinophilic chemotactic factor -A • N- Neutrophilic chemotactic factor -A	

Factors affecting Hypersensitivity 00:13:47

1. Genetic make up
   - Both parents are allergic -50% risk in the child
   - single parent allergic – 30% risk
2. Dose of allergen

TH1	TH2
↓ Interferon γ ↓ Hypersensitivity I is inhibited	↓ Interleukins – IL3, IL-4, IL-5 ↓ IgE mediated hypersensitivity

Manifestations: 00:15:52

1. Systemic Anaphylaxis – fatal reaction
   - Within few minutes
   - E.g.: drug allergies, bee sting, scorpion sting, nuts & sea food allergies
   - DOC – Adrenaline
2. Localized anaphylaxis
   - within days
   - less fatal
   - E.g.: Atopy
     - Asthma
     - Atopic dermatitis
     - Food & drug allergies

Diagnosis 00:18:02

1. Skin prick test
   - Suspected allergen is given s.c. or i.d.

     ↓

     Wheal & flare reaction
   - Less expensive
   - Risk of anaphylaxis
2. Radio immune sorbent test (RIST)
   - Quantitative test for total serum IgE
3. Radio immune allergen specific test (RAST)
   - Quantitative test for allergen specific IgE

Treatment

1. Avoid allergen
2. Hypo sensitization
    - Introducing allergen s.c. in increasing dose at regular intervals.
3. Drugs – Antihistaminic
    - Theophylline & Adrenaline
4. Consistent treatment is advised so continuous follow up is necessary.

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TYPE 2 HYPERSENSITIVITY REACTION

Type – 2 Hypersensitivity Reaction 00:18:00

- Mediated by IgG & rarely by IgM
- Antibodies react to antigens which are present in three forms

  1.Host cell surface Antigens

  e.g.: Blood Group Rh

  2. Exogenous antigen present on host cell surface

  e.g.: Drug coating RBC's

  3. Extracellular matrix antigen.
- Antigen + Antibody

  ↓

  Fc fragment of IgM brings about hypersensitivity reaction 2 by any of the three mechanisms

  1.Compliment mediated cytolysis

  2.Antibody dependent cellular cytotoxicity (ADCC)

  3.Auto Antibody cellular dysfunction (ADCD)

1. Compliment mediate cytolysis
   - Mediated by complements C5– C9which are activated by classical complement pathway
   - After activation cell lysis occurs

2.Complement mediated inflammation

Initiated by $\underline{c_{3a} \& c_{5a}}$ that leads to tissue injury.

↓

by product of complement activation

3.Bio mechanism → Opsonization

- By C3b – C4b
- They coat the target cells because of which phagocytosis is initiated

---

Complement mediated Hypersensitivity Reaction

00:06:35

- ABO incompatibility
  - lysis of donor RBC's due to antibodies produced by recipient to the donor antigens.
- Rh incompatibility
  - Rh – ve mother has anti –Rh antibodies due to prior exposure to Rh + ve blood.
  - This can cause lysis of fetal RBC after crossing placenta.
- Drug induced hemolytic anemia
  - Drug gets absorbed on RBC's → Antibodies + drug absorbed

↓

Antibodies produced

Antibodies + drug absorbed

↓

Lysis of RBC's

↓

Hemolytic anemia

  - e.g.: Black water fever in malaria after treatment with Quinine

- Auto immune hemolytic anemia, agranulocytes, thrombocytopenia
  - Auto antibodies produced against antigens on RBC's, granulocytes and platelets leading to autoimmune conditions.
- Pemphigus vulgaris
  - Auto antibodies on desmosome proteins

Antibody dependent cellular cytotoxicity (ADCC)

00:12:48

- Fab region of Antibody attached to Antigen/Target cell

↓

Destruction of target cells

- This happens to target cells that are too big for phagocytosis.
- While the empty Fc region of Antibody attached to NK cells

  or other effector cells via Fc receptor.

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Auto antibody Dependent cellular dysfunction (ADCD) 00:14:46

- Auto antibodies against self-antigens.

  e.g: Antibodies against receptors that leads to hyperactivation or inhibition.

↓ ↓

In grave's disease In myasthenia gravis

(Antibody against ACH receptor)

Other:

- Good Pasteur's syndrome
- Rheumatic fever
- Pernicious anemia

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TYPE 3 HYPERSENSITIVITY REACTION

Type 3 hypersensitivity reaction 00:00:20

- Antigen & antibody (Ag-Ab) complex mediated
- Excessive production of immune complexes leads to tissue injury
- In Ⓝ conditions

  Ag-Ab

  ↓

  Activates complement

  ↓

  Coated by complement

  ↓

  Phagocytosis

  - In chronic condition, autoimmune conditions (RA or SLE)
  - Excess Ag production
    - Causes increase in Ag – Ab complexes
    - This overwhelms the immune system which inhibits complete phagocytosis
  - Immune complex: soluble & insoluble
  - Antibody excess
    - Insoluble & large immune complexes are formed

      ↓

    - Localized type III hypersensitivity
  - Antigen excess
    - Soluble, small immune complexes are formed

      ↓

    - Generalized type III hypersensitivity

- Mechanisms by which immune complexes cause tissue injury

  1.Activation of mast cells
  - Produced via activated classical complement pathway – C3b, C5a→Degranulation of mast cells
  - Recruitment of neutrophils
    - Neutrophils are incapable of engulfing large complexes it starts to produce lytic enzymes that leads to tissue injury
    - This phenomenon is called frustrated phagocytosis

  2. Activation of platelets
  - Complexes bind to Fc receptors present on platelets

    ↓

    Thrombi formation & vasoactive amines releases

    ↓

    Tissue injury

  3.Activation Hageman factor

  ↓

  Release of Kinin

  ↓

  Edema & vasodilation

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Type III hypersensitivity

Localized

- Arthus reaction
- By insoluble Ag – Ab complexes
- Tissue necrosis because of vasculitis – seen at site of inoculation of Ag
- E.g., I bite desensitization bird fancier's disease

Generalized

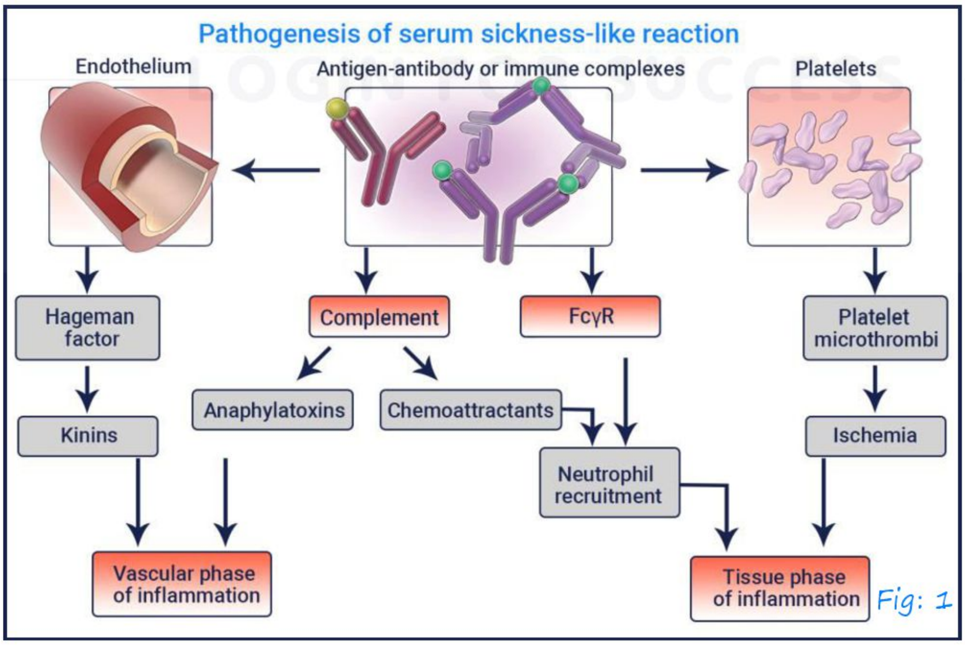
- Serum sickness
- Soluble & small Ag-Ab complexes so are transported to various organs by blood

↓

Inflammatory response

↓

- Serum sickness occurs after 7 – 8 days
- Symptoms: fever, lymphadenopathy, glomerulonephritis
- All the symptoms are due to deposition of immune complexes at various sites


Pathogenesis of serum sickness-like reaction
Endothelium
Antigen-antibody or immune complexes
Platelets
Hageman factor
Complement
FcγR
Platelet microthrombi
Anaphylatoxins
Chemoattractants
Kinins
Ischemia
Neutrophil recruitment
Vascular phase of inflammation
Tissue phase of inflammation
Fig: 1

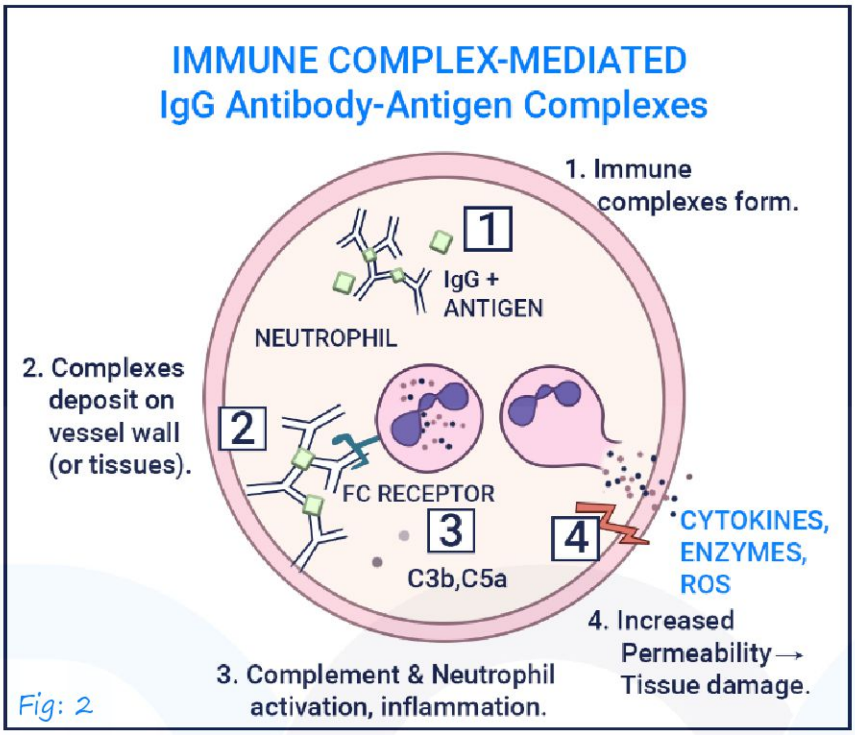

IMMUNE COMPLEX-MEDIATED
IgG Antibody-Antigen Complexes
1. Immune complexes form.
1
IgG + ANTIGEN
NEUTROPHIL
2. Complexes deposit on vessel wall (or tissues).
2
FC RECEPTOR
3
C3b,C5a
4
CYTOKINES, ENZYMES, ROS
4. Increased Permeability → Tissue damage.
3. Complement & Neutrophil activation, inflammation.

Fig: 2

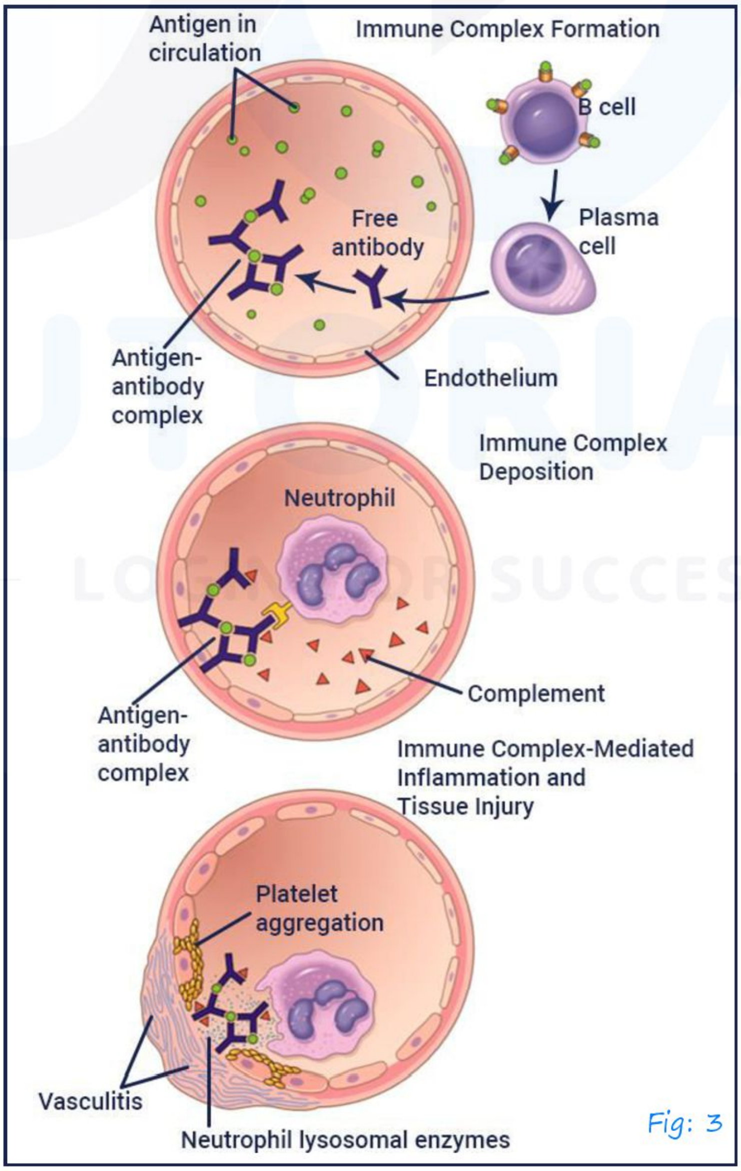

Antigen in circulation
Immune Complex Formation
B cell
Free antibody
Plasma cell
Antigen-antibody complex
Endothelium
Immune Complex Deposition
Neutrophil
Complement
Antigen-antibody complex
Immune Complex-Mediated Inflammation and Tissue Injury
Platelet aggregation
Vasculitis
Neutrophil lysosomal enzymes

Fig: 3

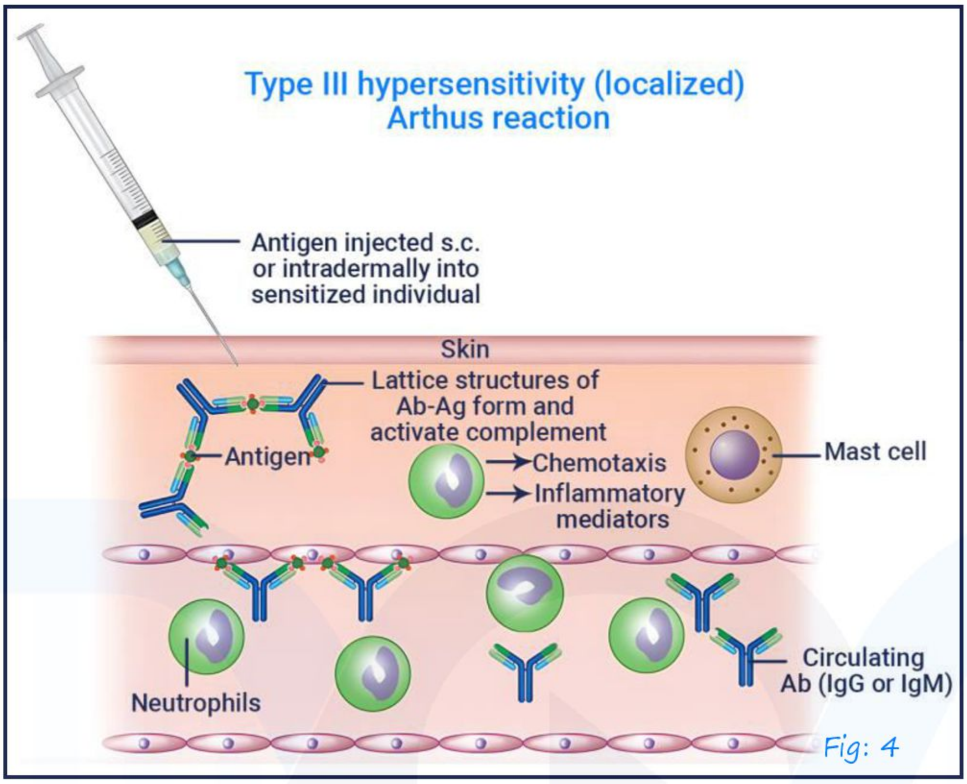

Type III hypersensitivity (localized)
Arthus reaction
Antigen injected s.c. or intradermally into sensitized individual
Skin
Lattice structures of Ab-Ag form and activate complement
Antigen
Chemotaxis
Inflammatory mediators
Mast cell
Neutrophils
Circulating Ab (IgG or IgM)

Fig: 4

- Serum sickness was common before because
  - Horse serum used as anti-tetanus serum (ATS)

    ↓ has

    Serum proteins that act as antigens

    ↓

    Immune response

    ↓

    Ag – Ab complex

    ↓

    Tissue injury

- Various conditions caused by type III hypersensitivity

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Connective tissue disorders:	Parasitic diseases:	Bacterial diseases:	Viral diseases	Others
• Due to autoantibodies forming immunocomplexes with self – antigens SLE (systemic lupus erythematosus) • Rheumatoid arthritis: Ab against human immunoglobulin • PAN (polyarteritis nodosa)	• Resulting from immunocomplex deposition Nephrotic syndrome in plasmodium malaria • Katayama fever in schistosomiasis • African trypanosomiasis	• Resulting from immunocomplex deposition • Streptococcus pyogenes: post streptococcal glomerulonephritis • Mycobacterium leprae (Lepra reaction type 2)	• With immunocomplex deposition • Hepatitis B (arthritis) • Infectious mononucleosis (Epstein – Barr virus) • Dengue (arthritis)	• Hyperacute graft rejection • Subacute bacterial endocarditis • Serum sickness

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TYPE 4 HYPERSENSITIVITY REACTION

Type – 4 hypersensitivity reaction 00:00:21

- Cell mediated – T_{DTH} cells (Delayed type hypersensitivity cells)
- Delayed hypersensitivity – after 48-72 hours
- Divided into 2 phases

1.Sensitization phase

- Antigen present is presented by antigen present cells (APC") (e.g., Langerhans cells] along with MHC – II to TH_2 cells

↓ divide into

TDTH cells

↓

Sensitized TDTH cells

2.Effector phase

- Sensited TDTH cells are subsequently exposed to Ag & releases various cytokines mainly INF – 8 & IL's

↓

Activates inflammatory cells (macrophages) at site of delayed hypersensitivity reaction

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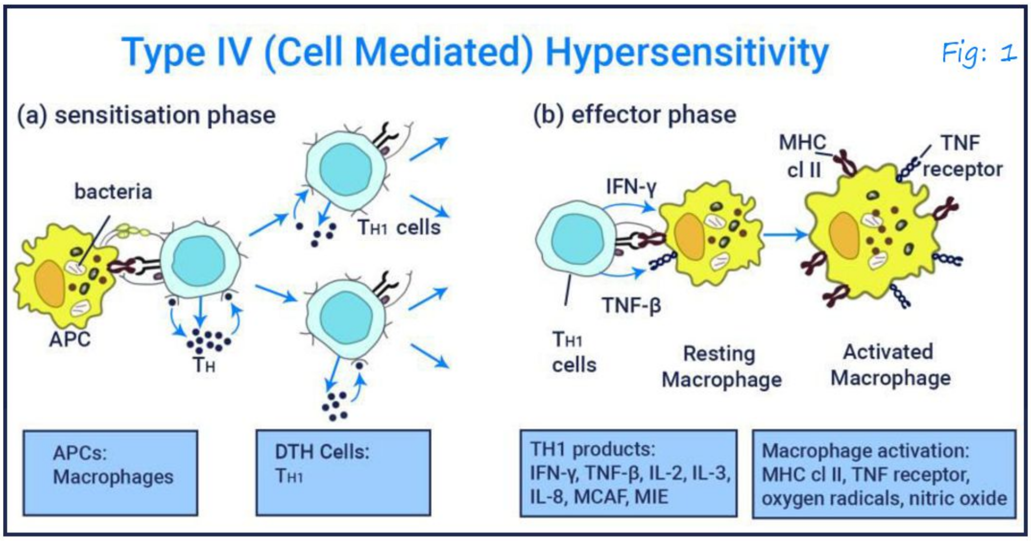
Type IV (Cell Mediated) Hypersensitivity
Fig: 1
(a) sensitisation phase
bacteria
APC
TH
TH1 cells
(b) effector phase
IFN-γ
MHC
cl II
TNF
receptor
TNF-β
TH1
cells
Resting
Macrophage
Activated
Macrophage
APCs:
Macrophages
DTH Cells:
TH1
TH1 products:
IFN-γ, TNF-β, IL-2, IL-3,
IL-8, MCAF, MIE
Macrophage activation:
MHC cl II, TNF receptor,
oxygen radicals, nitric oxide

Role of type - IV hypersensitivity 00:04:26

- Mainly of defense mechanism against intracellular micro – organisms

  ↓

  M. TB&M. Leprae

- Granuloma formation (in TB)

  - TH cells are replaced by macrophages

Divide into

Epithelial cells

Multinucleate giant cells

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- Tuberculin test

  - In a Sensited individual

    ↓

    T_{DTH} are present

  - When tuberculin antigen is given Intradermally

    ↓

    Erythema & induration after 48-72 hours

    (principle of tuberculin test

- Contact dermatitis
  - With nickel or poison oak (haptens)
  - Low Molecular weight molecules
  - Do not mount immune response

↓

  - Hapten + carrier = immune response (Can mount an immune response when hapten comes in contact with a carrier)
  - (Nickel + skin protein) (skin protein acts as a carrier)

↓

APC'S with MHC – II

↓

TH cells

↓ activates

Macrophages

↓ releases

Lytic substances

↓

Skin lesions

TUTORIALS
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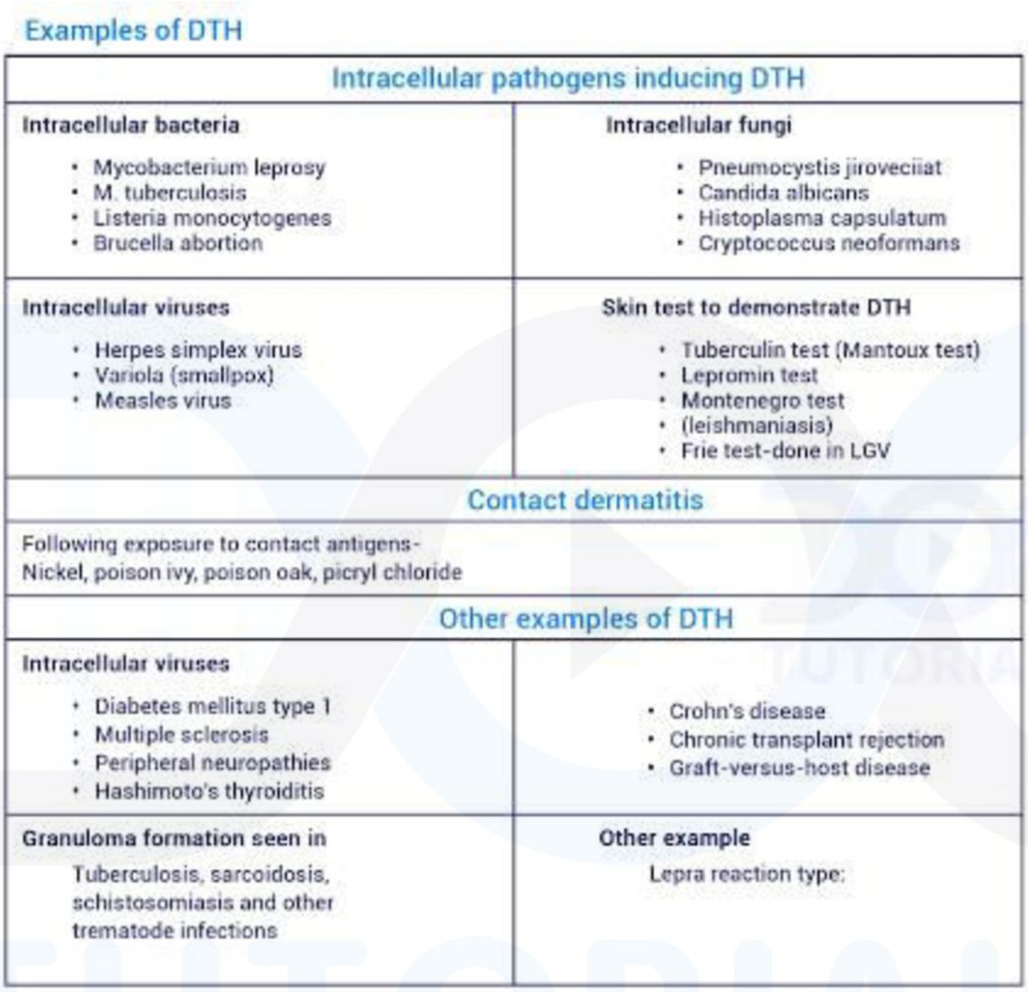
Examples of delayed type hypersensitivity

00:08:48



Examples of DTH

<table>
<tr><th colspan="2">Intracellular pathogens inducing DTH</th></tr>
<tr><td>Intracellular bacteria
• Mycobacterium leprosy
• M. tuberculosis
• Listeria monocytogenes
• Brucella abortion</td><td>Intracellular fungi
• Pneumocystis jiroveciiat
• Candida albicans
• Histoplasma capsulatum
• Cryptococcus neoformans</td></tr>
<tr><td>Intracellular viruses
• Herpes simplex virus
• Variola (smallpox)
• Measles virus</td><td>Skin test to demonstrate DTH
• Tuberculin test (Mantoux test)
• Lepromin test
• Montenegro test
• (leishmaniasis)
• Frie test-done in LGV</td></tr>
<tr><th colspan="2">Contact dermatitis</th></tr>
<tr><td colspan="2">Following exposure to contact antigens-
Nickel, poison ivy, poison oak, picryl chloride</td></tr>
<tr><th colspan="2">Other examples of DTH</th></tr>
<tr><td>Intracellular viruses
• Diabetes mellitus type 1
• Multiple sclerosis
• Peripheral neuropathies
• Hashimoto's thyroiditis</td><td>• Crohn's disease
• Chronic transplant rejection
• Graft-versus-host disease</td></tr>
<tr><td>Granuloma formation seen in
Tuberculosis, sarcoidosis, schistosomiasis and other trematode infections</td><td>Other example
Lepra reaction type:</td></tr>
</table>

TUTORIALS
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ESEHERICHIA COLI

- They are commensal present in human intestine & animals
- It is tested for during test for contamination of water.

↓

E. coli → survives for short time

↓

Aerotolerant E. coli (44^o)

↓

Early contamination of water

Virulence factors: 00:01:40

1) Surface antigens

Somatic Antigen	Capsular Antigen	Fimbrial Antigen	Flagellar Antigen
• 'O' antigen • It is associated with LPS • Virulence factor showing endotoxic activity	• 'K' antigen • Only in few strains • Makes 'O' antigen inagglunitable to 'O' antisera	• CFA- associated with EPEC (Centro pathogenic E. coli) • P. fimbriae → P antigen on RBCS	• It is mainly for locomotion

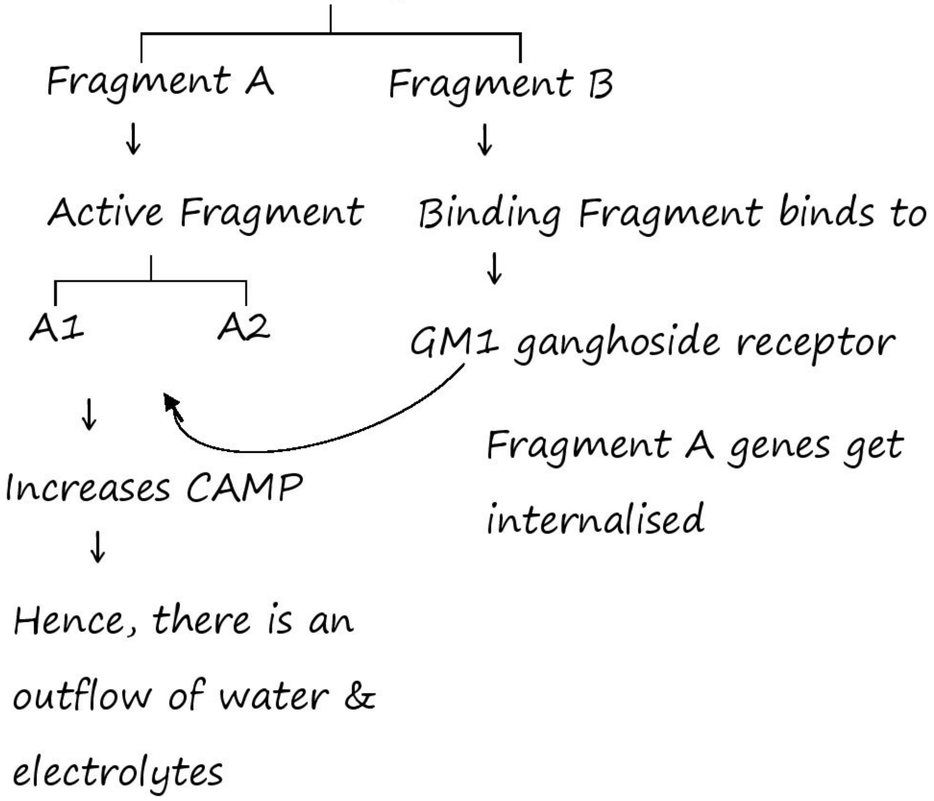
Mainly to inhibit phagocytosis

2. Toxins

- Hemolysis
- Enterotoxin

A. Heat Labile toxin (LT)

- Produced by EPCE
- It resembles cholera toxin
- It has 2 fragments



Fragment A
Fragment B
Active Fragment
Binding Fragment binds to
A1
A2
GM1 ganghoside receptor
Increases CAMP
Fragment A genes get internalised
Hence, there is an outflow of water & electrolytes

B.Heat stable toxin

- Produced by EPEC
- This has 2 fragments / components

S T1

Acts by ↑ c GMP

S T2

- Unknown mechanism
- Either due to

↑ CAMP

(or)

↑ GMP

Tests

Invitro

Heat Labile → a.Ligated illeal loop test

→ +ve (18hrs)

ST → +ve (6hrs)

Invivo tests

a. Production of steroid in mouse adrenal culture

b. Tissue culture method – positive

c. Tissue culture method – negative (for ST)

d. Eiken test

C.Verocytotoxin / Shiga – like toxin

- Produced by EHEC
- This has 2 Fragments

A fragment

↓

Active fragment

MDA → inhibition of protein synthesis

B fragment

↓

Binding

- Tests used to determine the toxins produced
  - Latex agglutination
  - ELISA
  - Molecular methods

Clinical magnification: 00:14:30

1. UTI – Uropathogenic E. coli (UPEC)
2. Diarrhea
   a. EPEC
   b. ETEC
   c. EIEC
   d. EHEC
   e. Diffuse adhesive E. coli
3. Peritonitis
   a. Primary – spontaneous
   b. Secondary
4. Meningitis
5. Endocarditis
6. Soft tissue & wound infections
7. Immunocompetent people

Lab diagnosis: 00:16:27

Sample type - Urine, PUS, blood, or any exudate

↓

Gram staining – GNB with pus cells

↓

Hanging drop – Motile organism & has peritrichous flagella

Culture

- Blood agar
  - Grey, moist colonies With or without Hemolysis (UPEC)
- MAC – pink lactose fermenting colonies

  ↓

  Flat colonies
- (LED – medium

Biochemicals

- Catalase → +ve
- Oxidase → -ve
- Indole → +ve
- Citrate → -ve
- Urease → -ve
- TSI → A/A with gas
- Sugars – ferments all sugar except sucrose
- MR +ve
- VP -ve

Molecular methods

- PCR
- Multiplex PCR

Uropathogenic E. coli 00:21:45

- Predisposing factors
  - Common in females due to short urethra & closer to anus
  - Pregnant – due to stasis of urine
  - Urinary stasis due to prostate enlargement & caliculi

- Collection
  - Suprapubic aspiration (Best methos)
  - Collect mid-stream uric
- Transport
  - b/w 1-2 hours → Refrigerate up to 24hrs but requires glycerol or boric acid to prevent commensals from growth
- Direct examination
  - Pus cells > 8mm pus cells /mm^3
  - Nitrate reduction test
  - Leukocyte staining
  - Catalase test
  - Culture

Kass Concept

- > 10^5 CFU/ml – significant bacteriuria (CFU – colony forming unit)
- Lower counts significant in
  - Pyelonephritis
  - Pt. on antibodies
  - Suprapubic aspiration sample

Diarrhea causing E. coli: 00:27:28

Enteropathogenic E. coli (EPEC)	**Enterotoxigenic E. coli (ETEC)**
• Causes infantile diarrhea • MOA → due to pili which causes cup - like structures called pedestals • Non-toxogenic non-invasive	• Known as traveller's diarrhea • MOA Binding with CFA Toxins - ST - ↑ CGMP - LT - ↑ CAMP ↓ Loss of water & electrolytes • Toxogenic but non - invasive

Entero invasive E. coli [EIEC]	**Enterohaemorrhagic E. coli [EHEC]**
• MOA – invasion of epithelium of intestine • It produces symptoms like shigellosis ↓ Diarrhea associated with blood & mucus	• MOA – Due to inhibition of protein synthesis (Shiga toxin or Verocytoxin) • It produces hemorrhagic colitis & hemorrhagic uremic syndrome • Fever is absent • Culture - Rainbow Agar→Procedures black colonies - Sorbitol Mac Conkey Agar→ Produces pale coloniles→ Sorbitol not fermented

Entero aggregative E. coli (EAEC)	**Diffuse Adhesive E. coli (DAEC)**
Adherence To Hep – 2 – Brick stacked appearance	Diffused Diarrhea in children (2 -6 yrs)

CLOSTRIDIUM

Clostridium 00:00:11

- Bacillus does not have bulging spores
- Clostridium has bulging spores
- Clostridium – Gram positive rods, bulging spores
- A important species pathogenic to man

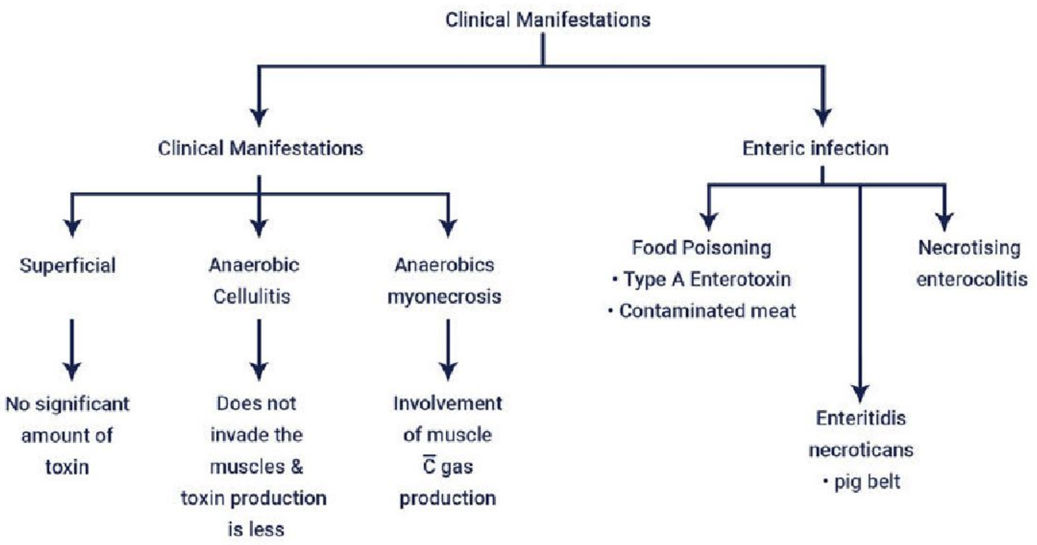
  - Cl. Perfringens → gas gangrene (60%)
  - Cl. Tetani → tetanus
  - Cl. Botulinum → Botulinism
  - Cl. Difficile → submembranous colitis
- Clostridium are saprophytes

  - Decaying matter
  - Soil
  - Human intestine

Clostridium perfringens 00:02:40

- Gram positive bacilli sub-terminal spores but in culture → does not produce any spores
- Virulence factors

  - 4 major toxins
  - 4 minor toxins
  - Heat labile enterotoxin
  - Soluble substances
    - Neuraminide
    - Histamine


Clinical Manifestations
Clinical Manifestations
Enteric infection
Superficial
Anaerobic Cellulitis
Anaerobics myonecrosis
No significant amount of toxin
Does not invade the muscles & toxin production is less
Involvement of muscle $\bar{C}$ gas production
Food Poisoning
• Type A Enterotoxin
• Contaminated meat
Necrotising enterocolitis
Enteritidis necroticans
• pig belt

Gas gangrene 00:07:00

- It is diffuse myonecrosis
- Pathogenesis
  - Anaerobic environment
    - RTA with crush injuries

      ↓

      Devitalised tissue & ischemia (+)
  - Spores of clostridium

Clinical features

1. Crepitus
2. Brawny edema
3. Foul smelling serosanguinous discharge
4. Severe pain

Lab diagnosis

- Specimen- devitalised tissue
  - Exudate from inside of the lesion

Gram staining
- Box car shaped GPB with sub-terminal spores

Culture
- RCM
- Egg yok agar

↓

Anaerobically

Blood agar
- Target haemolysis

↓

A zone of complete haemolysis surrounded by incomplete haemolysis

complete haemolysis → o toxin

incomplete haemolysis → α toxin

Nagler's reaction

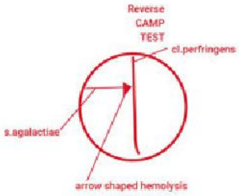
- Egg yok agar
- Agar containing serum

Heat tolerance test

- Cl perfringens

↓

- RCM – 4-6hrs at 45°C
- Litmus milk test – stormy clot appearance
- Reverse camp test

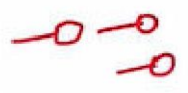

Reverse
CAMP
TEST
cl.perfringens
s.agalactiae
arrow shaped hemolysis

Treatment

- Surgical debridement
- Antibiotics
- Hyperbaric oxygen
- Antitoxin

Clostridium tetani 00:17:15

- Description – gram positive bacilli with terminal spore



Drum Stick Appearance

- Isolated in soil
- Seen in hospital environment, intestine of humans & animals

Virulence factors

- Tetanolysin – oxygen & heat labile
- Tetanospasmin – oxygen stable & heat labile

Toxin binds to motor neural terminal

↓

Inhibits the release of pre-synaptic inhibitory neurotransmitters like GABA & Glycine

↓

Spastic contraction of muscles

Transmission

- Injury
- Aseptic practices
- Delivery – neonatal tetanus
- Otitis media-otogenic tetanus

Clinical manifestation

- 1st symptom
  - Trismus or lock jaw
  - Exaggerated deep tendon reflexes
  - Descending spastic paralysis

Complications

- Risus sardonicus
- Opisthotonos → due to spastic contraction of extensor muscles

Lab diagnosis

- Gram staining – GPB with terminal spores
- Culture – RCM – Meat particles turn black in colour
  - Blood agar with polymyxin – **swarming**

AllaTafheem.com

Treatment

- Active immunisation
  - Human tetanus immunoglobulin
  - ATS (equine)
- Prevention of tetanus (passive immunisation)
  - Monovalent vaccine (toxin in formaldehyde)
  - DPT vaccine

DPT

- In children – 4,10,14,16-24months, 5Years
  10yers & 16 years
- In adults – 4 doses

2 doses 1 month apart & then 1 year & 6 years

Category

- A- 5 yrs
- B- 5-10years
- C- >10 years
- D- Not received the course or boost

In a simple wound	Other wound
A- nothing required	Nothing
B- 1 dose of toxoid	1 dose of toxoid
C- 1 dose of toxoid	1 dose of toxoid with human immunoglobulin
D- course + immunoglobulin	Course + Immunoglobulin

Neonatal tetanus 00:27:45

- Between 3-28 days – neonatal tetanus
  ↓
  Baby is unable to cry/suckle
- 8th day disease

- Previous associated with dung being put on umbilical cord
- Decrease Tetanus due to
  - Clean practices – 5 "C"
  - Vaccination of pregnant women in 2nd trimester

Clostridium Botulinum 00:30:00

- Causes botulism
- Cl botulinum – GPB with subterminal, oval bulging spore
- Botulinum toxin – highly toxic high threat potential

↓

Acts on Ach receptors

↓

In NMJ

↓

Inhibits release of Ach

↓

Causes flaccid paralysis

Serotypes – 8 serotypes

- A,B,C1,C2,D,E,F,G

Clinical conditions

- Food botulism
  - ↓ Canned home food
- Wound botulism
  - ↓ Spores affecting the wound
- Infant botulism
  - ↓ Less than 1 year
  - ↓ Floppy infant syndrome
- Iatrogenic botulism
  - ↓ Overdose of botulinum toxin

Dairy products

- Vomiting < 6 hrs – staphylococcus food poisoning
- Ground beef – enterohaemorrhagic enterocolitis (EHEC)

Lab diagnosis

- Gram staining
  - GPB with subterminal oval spores
- Cultures
  - RCM
    - Proteolytic changes - foul-smelling

      A, B, F, G
    - Saccharolytic

      C1, C2, D, E
  - BLOOD agar – semi-transparent colonies with fimbriate border

Treatment

- Respiratory support
- Antitoxin
- Wound botulism → surgical debridement

Clostridium difficle 00:38:18

- It causes pseudomembranous colitis
- Obligate, anaerobe, spore bearing

Risk factor

- Increase hospital stay → causes colonisation of spores
- Acute onset of diarrhoea
- Antibiotic overuse – Cephalosporin
  - Ampicillin
  - Fluoroquinolone

Pathogenesis

- Toxin mediated
  - Toxin A
  - Toxin B

Clinical symptoms

- They present with diarrhoea, without blood
  - h/o prolonged antibiotic use
  - Old age
  - Prolonged hospital stay
  - Pseudomemnrane – plaque

↓

contains necrotic leukocytes, cell debris, mucus

Lab diagnosis

A. Culture

- CCYA – cefoxitin cycloserine egg yolk ager
- CCFA – Cefoxitin cycloserine fructose ager

↓

Anaerobically at 37°C for 48hrs

B.Toxin A/B determination

- Rapid test
- Glutamate dehydrogenase antigen demonstration in stool
- PCR- identifies genes causing cl. Difficile related diarrhoea

Treatment 00:04:42

- Stop the offending antibiotics
- Decrease Hospital stays
- Drug therapy – Metronidazole
  - Vancomycin

CLINICAL PARASITOLOGY

Hymenolepis nana

- Smallest Cestode

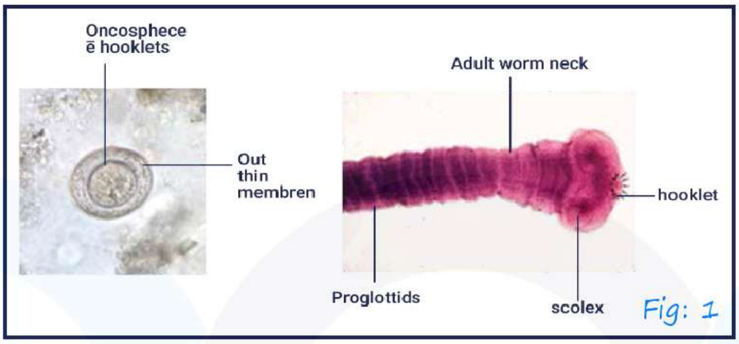

Oncosphece
ē hooklets
Out
thin
membren
Adult worm neck
hooklet
Proglottids
scolex

Fig: 1

- Dwarf tape worm
- Definitive host - Man
- Habitat - Proximal ileum
- Infective form & mode of infection - Eggs

Life cycle: In Man

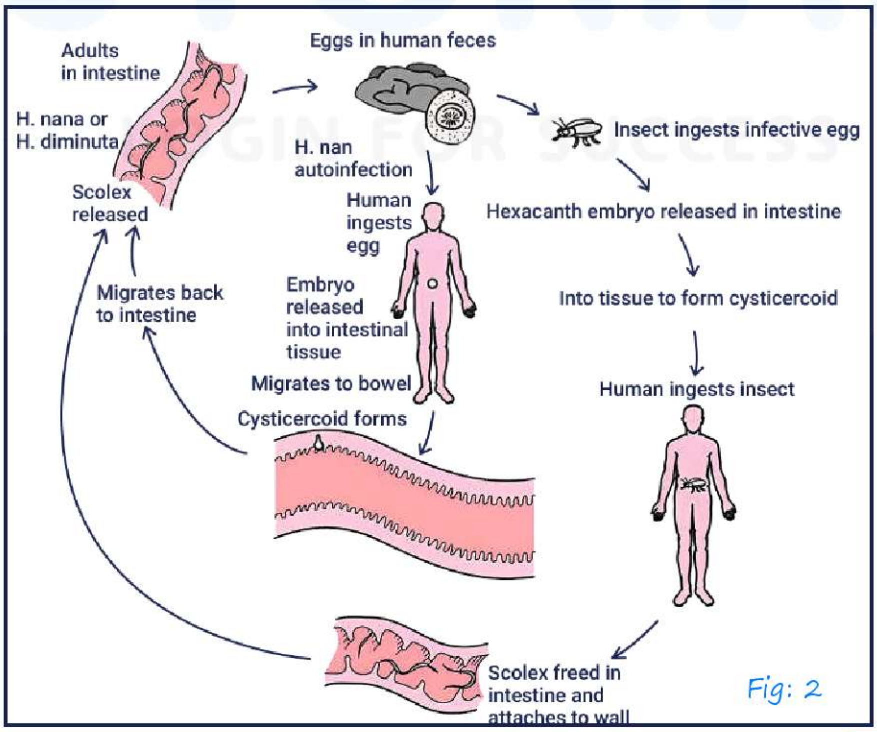

Adults
in intestine
H. nana or
H. diminuta
Scolex
released
Migrates back
to intestine
Eggs in human feces
H. nan
autoinfection
Human
ingests
egg
Embryo
released
into intestinal
tissue
Migrates to bowel
Cysticercoid forms
Insect ingests infective egg
Hexacanth embryo released in intestine
Into tissue to form cysticercoid
Human ingests insect
Scolex freed in
intestine and
attaches to wall

Fig: 2

- Treatment - Praziquantel
- Lab diagnosis - Demonstration of eggs – Oncosphere / hooklets

TUTORIALS
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TREMATODES

Trematodes 00:00:10

General characteristics

- Leaf shaped
- Unsegmented

} Trematodes

Classification based on habitat

- Blood flukes
- Intestinal flukes
- Liver flukes
- Lung flukes

TUTORIALS

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General features

- Oral sucker & ventral suckers
- Circulatory system is absent
- CNS – rudimentary
- Hermaphrodites – except schistosomes

Schistosomiasis

- S. haematobium → urinary bladder
- S. mansoni → sigmoid rectal
  - Interior mesenteric vein
- S. japonicum → ileocecal
  - Superior mesenteric vein
- S. mekongi
- S. intercalactum → "Acid fast"

S. haematobium

- Adult worm – male & female worm → female worm is longer
- Egg: Non – operculated eggs
  - Yellow – brown in color
  - Terminal spine

Life cycle

- Infective form – cercaria

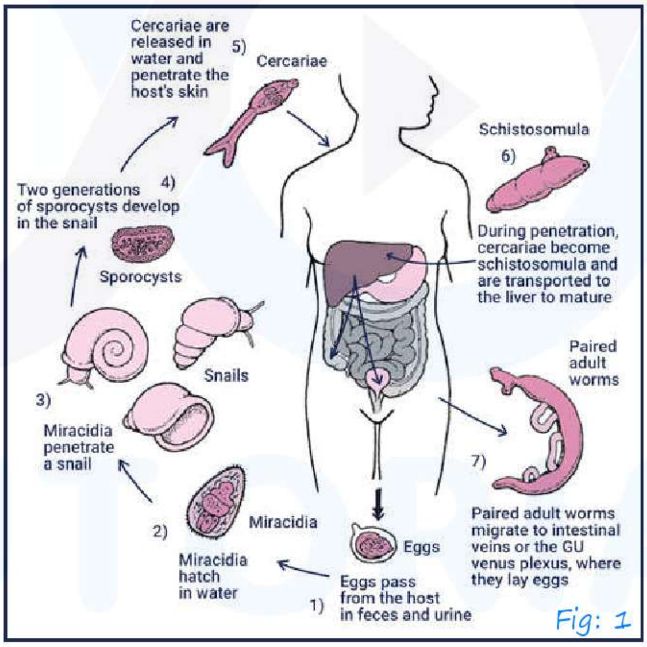
Cercariae are released in water and penetrate the host's skin
5)
Cercariae
Schistosomula
6)
Two generations of sporocysts develop in the snail
4)
During penetration, cercariae become schistosomula and are transported to the liver to mature
Sporocysts
Paired adult worms
Snails
3)
Miracidia penetrate a snail
7)
2)
Miracidia
Paired adult worms migrate to intestinal veins or the GU venus plexus, where they lay eggs
Eggs
Miracidia hatch in water
1)
Eggs pass from the host in feces and urine

Fig: 1

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Clinical features

Acute

- Swimmer's itch
- Penetration of cercaria

Chronic

- Urinary bladder carcinoma
- Eggs → antigen → Mucosal irritation
- Calcified eggs
- Symptoms-
  - Painless hematoma
  - Proteinuria

Lab diagnosis

Demonstration of egg	Antigen detection	Demonstration of antibodies	Intradermal test	Imaging test
↓ Egg with terminal spine	↓ CAA CCA	• CFI • HAI • ELISA	• Fairley's test	• USG • X -ray: calcified egg • IVP

- Treatment: Praziquantel → DOC

S. mansoni

- Definitive host- Man/mammals
- Intermediate host – snail
- Infective form – folk shaped cercaria

Clinical features

- Dermatitis ↓ Swimmer's itch
- Katayama fever ↓
  - Serum sickness like illness
  - Antigen – Ab complex
- Micro abscess formation

DOC TUTORIALS LOGIN FOR SUCCESS

Lab diagnosis

Demonstration of egg	Rectal biopsy	Imaging	Blood examination	Serological
↓ Lateral spine non – operculated	↓ Egg in mucosa	↓ USG X ray	↓ Eosinophilia	• Antigen • Ab

- Treatment: Praziquantel (DOC)

S. japanoicum 00:13:52

- Seen in Japan, China, Philippines
- Habitat: superior mesenteric vein
- Definitive Host – man
- Intermediate Host – snail
- Infective form – fork shaped cercaria

DOC TUTORIALS
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Clinical features

Katayama disease
↓
Severe
Dysentery

Chronic
Clay pipestem fibrosis
↓
Periportal fibrosis of liver

Trematodes causing dysentery:

- S. mansoni
- S. japonicum
- S. mekongi
- S. intercalatum

- Treatment: Praziquantel

TUTORIALS
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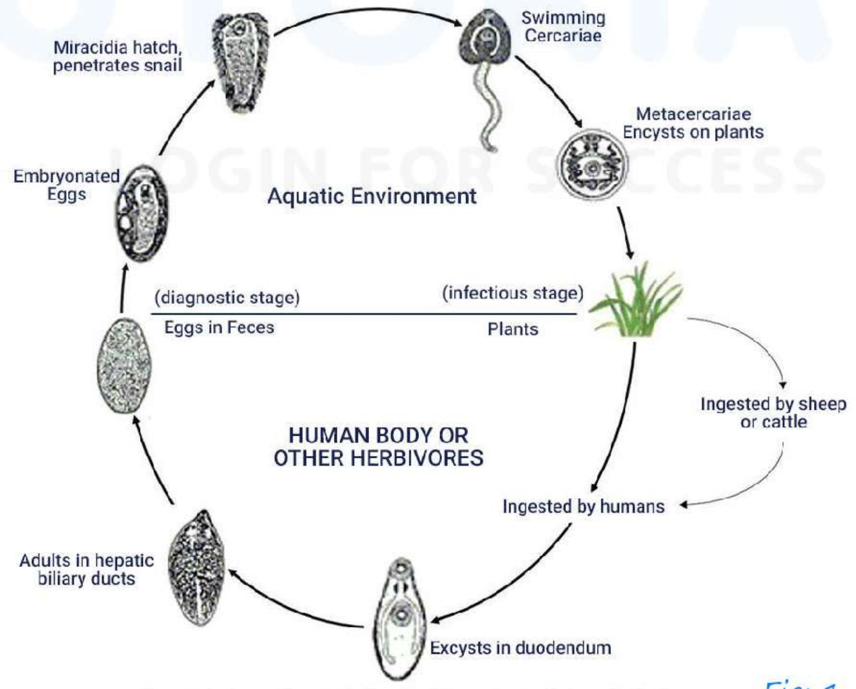
LIVER FLUKES

Liver flukes 00:00:10

Fasciola hepatica

- No malignant potential
- Sheep liver fluke
- Ovoid, operculated bile-stained egg
- Definitive host – Sheep > man
- Intermediate host
  - 1st Snail
  - 2nd Aquatic plant
- Mode of infection – Consumption of aquatic plants encysted by metacercaria

Life cycle


Miracidia hatch, penetrates snail
Swimming Cercariae
Metacercariae Encysts on plants
Embryonated Eggs
Aquatic Environment
(diagnostic stage)
Eggs in Feces
(infectious stage)
Plants
Ingested by sheep or cattle
HUMAN BODY OR OTHER HERBIVORES
Ingested by humans
Adults in hepatic biliary ducts
Excysts in duodendum

Fasciola hepatica Life Cycle, Trematode (Liver fluke) Fig: 1

Clinical features

- Acute – Pain abdomen, hepatosplenomegaly, fever
- Chronic – Biliary obstruction, cholelithiasis, obstructive jaundice

Lab diagnosis

- Demonstrates of egg – Ovoid, bile-stained egg
- Blood picture – Eosinophilia
- Serology – Antibodies
- Imaging

Doc in treatment = triclabendazole

Clonaris seneris / Chinese liver fluke

- Responsible for cholangiocarcinoma
- Chinese fluke
- Flask – shaped egg
- Habital – biliary duct & pancreatic duct

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DOC TUTORIALS LOGIN FOR SUCCESS

Eggs

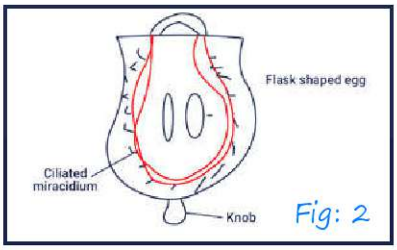

Fig: 2

- Intermediate host
  - 1st snail
  - 2nd fish

Life cycle

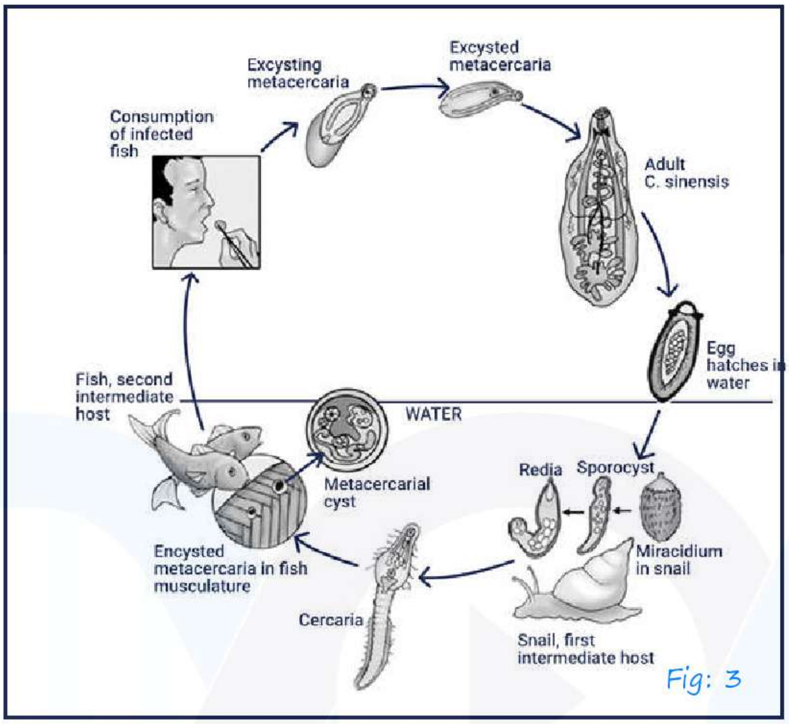
Excysting metacercaria
Excysted metacercaria
Consumption of infected fish
Adult C. sinensis
Egg hatches i water
WATER
Fish, second intermediate host
Metacercarial cyst
Redia
Sporocyst
Miracidium in snail
Encysted metacercaria in fish musculature
Cercaria
Snail, first intermediate host

Fig: 3

Clinical features

- Acute - fever, allergies
- Chronic - Cholangiocarcinoma

Lab diagnosis

- Demonstrates egg
  - Flask shaped with knob &
  - Ciliated miracidium
- Serology- antibodies
- Treatment – Praziquantel

Prevention

- Properly cooked fish
- Control of snails
- Proper disposal of feces

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LUNG FLUKES

Paragonimus westermani

- Habitat – cystic spaces connecting the bronchi (lung)
- Definitive host – Man
- Intermediate host:
  - 1st – snail
  - 2nd – crayfish

Clinical features

Pulmonary

- Hemoptysis
- Eosinophilia
- Cough
- Chronic TB

Extra –pulmonary

- Abdominal
  - Epigastric pain
  - Diarrhea
- Cerebral
  - Seizures
  - Headache
  - Neck stiffness

TUTORIALS
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Life cycle

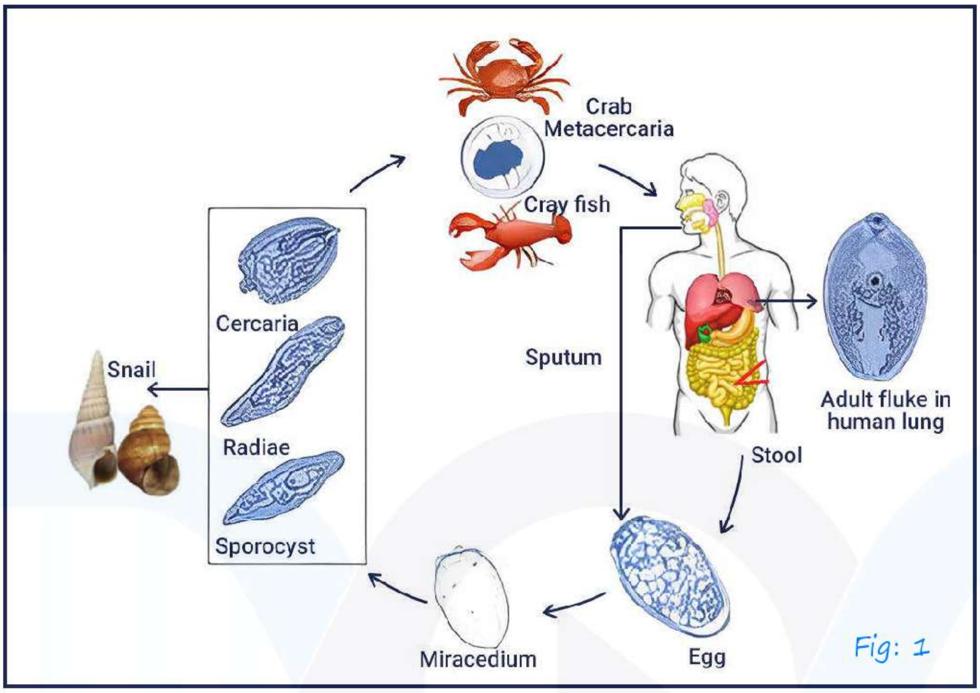
Crab
Metacercaria
Cray fish
Sputum
Adult fluke in human lung
Stool
Egg
Miracedium
Sporocyst
Radiae
Cercaria
Snail

Fig: 1

Lab diagnosis

Demonstration of egg
- Sputum & stool

Serological- antibodies
↓
- CFI
- ELISA

Imaging
X ray – TB
↓
CT scan → lung & brain

- Treatment – Praziquantel

TUTORIALS
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INTESTINAL FLUKES

- Habitat – Duodenum & jejunum of man, pig (reservoir)
- Definitive host – Man, pig
- Intermediate host
  - 1st – snail
  - 2nd – aquatic plants
- Infective form – Metacercaria encysting aquatic plants
- Clinic features
  - Epigastric pain
  - Diarrhea
  - Protein loss enteropathy
  - Malabsorption
  - B12 deficiency

TUTORIALS
LOGIC FOR SUCCESS

Life cycle

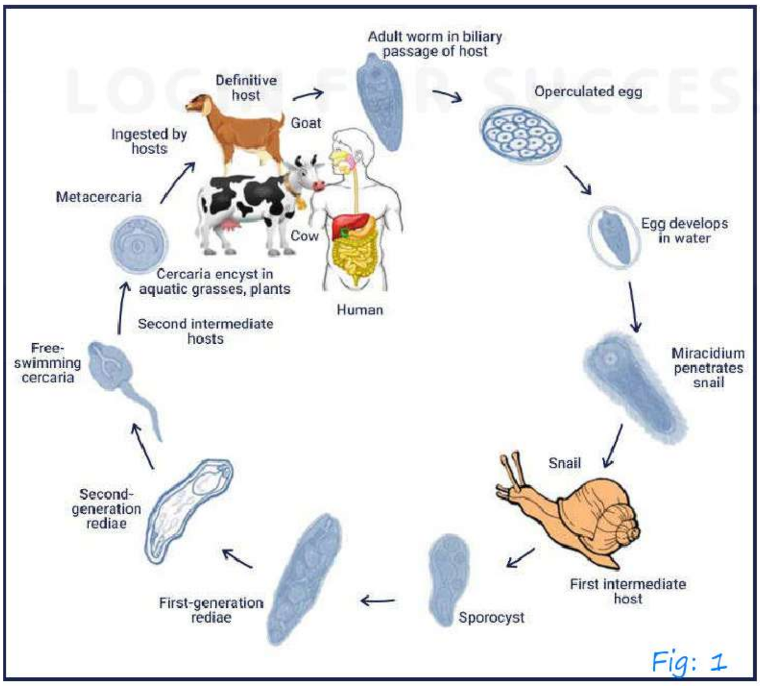
Adult worm in biliary passage of host
Operculated egg
Egg develops in water
Miracidium penetrates snail
Snail
First intermediate host
Sporocyst
First-generation rediae
Second-generation rediae
Free-swimming cercaria
Second intermediate hosts
Cercaria encyst in aquatic grasses, plants
Metacercaria
Ingested by hosts
Definitive host
Goat
Cow
Human

Fig: 1

Lab diagnosis
- Egg –Operculated egg – Ovoid, Bile stained
- Serological – Antibody – ELISA, CFT

Treatment: Praziquantel

TUTORIALS
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